Soil Phosphorus Dynamics and Bioavailability in New Zealand Forest Ecosystems

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Abstract of a thesis submitted in partial fulfilment of the requirement for the Degree of Doctor of Philosophy

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By Ivan Chirino-Valle

Phosphorus (P) is an essential element for all living organisms and the productivity of natural and managed ecosystems is generally limited by the bioavailability of P in soil. Previous studies showed that significant changes in soil P occurred following a land-use change from grassland to short-rotation plantation forestry in hill and high country areas of New Zealand. However, most of these studies involved paired-site comparison at a single point in time after forest planting (commonly 10-20 years), and our understanding of when the changes in soil P occurred and the mechanisms involved is limited. The main objective of this study was to investigate temporal changes in the nature and bioavailability of soil P associated with forest development, including the effect of different tree species. This involved conducting five major experiments over a range of timescales from seasonal to millennia at four sites in New Zealand (Orton Bradley Park, Glendhu, Lincoln, Haast) using a variety of techniques to quantify temporal changes in the nature and bioavailability of soil P. The results of this research clearly demonstrated that dramatic changes in the nature and bioavailability of soil P occurred during the first 5 years following tree planting, which resulted in significant mineralisation of soil organic P. This in turn was mainly attributed to a combination of factors including P acquisition by trees and reduced quantities of organic matter and P returned to soil as a consequence of the cessation of grazing. Surprisingly, the initial changes in soil P were similar under three contrasting tree species (*P. radiata*, *C. macrocarpa*, *E. nitens*), which indicated that these tree species accessed similar forms of P in soil despite differences in mycorrhizal associations and growth. This was confirmed in results from the seasonal study carried out on the same site, while the relative short-term bioavailability of P in soils taken from the Haast native forest chronosequence was similar for *P. radiata pine* and *C. macrocarpa*. It was also shown that net mineralisation of organic P occurred when trees were planted in high organic matter/low P fertility soils, while

net immobilisation of P (i.e. increases in organic P) occurred when trees and pasture were established simultaneously in a low organic matter/high P fertility soil. Increases in recalcitrant soil P indicated a shift towards more stable P forms with forest development, which was associated with the increased inputs of more recalcitrant organic matter and P with time.

Keywords: Soil phosphorus bioavailability; soil organic phosphorus mineralisation; soil phosphorus fractionation; *Pinus radiata*; *Cupressus macrocarpa*; *Eucalyptus nitens*.

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CHAPTER 1 INTRODUCTION

Phosphorus (P) is an essential element for all living cells because it is a structural component of nucleic acids, co-enzymes, phospho-proteins, phospholipids, and determines many metabolic processes (provides energy as ADP and ATP). Due to the essential nature of P to living organisms, the low solubility of natural P-containing compounds, and the slow natural cycle of P, primary production is generally limited by P availability in soil and consequently P deficiency represents a major constraint to productivity at the ecosystem level (Bünemann and Condron, 2007; Richardson et al., 2005).

The New Zealand land mass was thrust up from the ocean 120-140 million years ago and as a consequence of its location on the junction of the major Pacific and Indian-Australian tectonic plates, New Zealand is geologically very active. Between 50 and 25 million years ago New Zealand was gradually pushed down until only 20% of the current land area was above sea level. Thereafter the land was raised and twisted by plate movement, a process that is continuing with an uplift rate of up to 10 mm per annum. The impacts of this tectonic stress are evident in the nature of the New Zealand landscape. Only 15% of the land area is flat (<3º), while rolling (3-12º), hill (12-28º) and steepland (>28º) areas make up 14, 21 and 48%, respectively, while 60% of the land is more than 300 m above sea level (20% above 900 m).

When Maoris first arrived in New Zealand c. 1200 most of the land was mainly covered by dense forest, which they began to clear by burning to facilitate settlement and hunting. Most of the forest clearance occurred between 1350 and 1550 and the consequent long-term impacts were greatest in the drier east coast regions of both main islands. By 1840 (immediately prior to large scale European settlement) the forest cover had been reduced from 23 million ha to 14 million ha.

Deforestation continued under European settlement as land was cleared for pastoral agriculture. Accordingly, remaining native forest cover now represents only 23% of the total land area (6.3 million ha), although most of this forest is now held in the conservation estate.

Currently 16 million ha of land in New Zealand is utilized for agriculture, horticulture and plantation forestry. Most of this area (12 million ha) is under extensive pastoral farming (sheep and beef) on hill country and steepland, while 2 million ha of flat and rolling land is under intensive dairying, arable cropping and horticulture. Plantation forestry is based on growing exotic trees (principally radiata pine (*Pinus radiata*) from North America) on a 20-40 year rotation. Recent dramatic changes in land-use in New Zealand can be attributed to a combination of factors including the removal of all product and input subsidies in 1985. Thus between 1985 and 1995 sheep numbers declined from 70 to 50 million (<40 million by 2012), and while total cattle numbers remained static over this period at 9 million the number of dairy cows increased by 40% from 2.9 to 4.1 million. Over the same period there was a dramatic increase in the land area under plantation forestry from 0.9 million ha in 1985 to 1.5 million in 1995 (increased further to 1.8 million ha by 2000). Most of the recently established forests have been planted on hill country and steepland developed under pastoral farming since European settlement, and occurred in response to continued declines in returns from meat and wool and expected higher returns from forest products in the future.

Native and managed plantation forests account for one third of the land area in New Zealand and therefore play a vital role in the country's ecological, environmental and socio-economic prosperity and sustainability. These forests also provide a unique template to investigate and quantify the key properties and processes that drive soil nutrient dynamics and bioavailability, including P. For example, a series of native forest ecosystem development sequences have been identified and studied in New Zealand, including the glacial retreat system at Franz Josef (Richardson et al., 2004) and the coastal fore-dune ridge system at Haast (Turner et al., 2012). Differences in the amounts and forms of soil P in these chronosequences have been used to

advance our fundamental understanding of P cycling and bioavailability (Allison et al., 2007; Eger et al., 2013; Turner et al., 2007a; Turner et al., 2013). In addition, the recent dramatic changes in land-use linked to the afforestation of grazed pasture in New Zealand noted above provide an ideal opportunity to investigate how contrasting plant species and land management practices affect the amounts, nature and bioavailability of soil P (Chen et al., 2008).

CHAPTER 2 LITERATURE REVIEW

2.1 Phosphorus Cycling and Bioavailability in Soil-Plant Systems

The major pools of P on earth are the oceans and the lithosphere, with < 1% is found in the terrestrial ecosystems compartments: atmosphere, plant biomass and soil (Stewart et al., 2005). Most of the P in terrestrial ecosystems is present in soils, which generally contain between 100-3000 kg ha^{-1}, but only a small proportion of this (<1%) is immediate available to plants at any time (Bünemann and Condron, 2007; Condron and Tiessen, 2005; Richardson *et al*., 2005). The primary source of P in soils is the sparingly soluble calcium phosphate apatite (Newman, 1995; Walker and Syers, 1976). 'Labile P' refers to the soil P that is in equilibrium within the soil solution (plant available), while P forms that have slower equilibrium are termed 'non-labile' (Pierzynski *et al*., 2005).

Phosphorus cycling or dynamics in soil can be defined as a series of processes influenced by the nature of the inorganic and organic solid phases present, the type and intensity of biological activity, the chemistry of the soil solution (pH, ionic strength, redox potential), and abiotic factors like texture and moisture content, (Bünemann and Condron, 2007; Pierzynski *et al*., 2005; Quiquampoix and Mousain, 2005) (Figure 2.1).

In general terms, the P cycle in the soil begins when primary apatite P in rock breaks down due to physical and chemical weathering releasing P. Once in solution, the P incorporated into the system is converted to secondary Pi and organic P forms, some of which are of limited availability to plants and microbial communities (Walker and Syers, 1976). These forms can be affected by chemical and biochemical processes including sorption-desorption (oxidation-reduction) and mineralization-immobilization (Condron and Tiessen, 2005; Pierzynski et al., 2005).

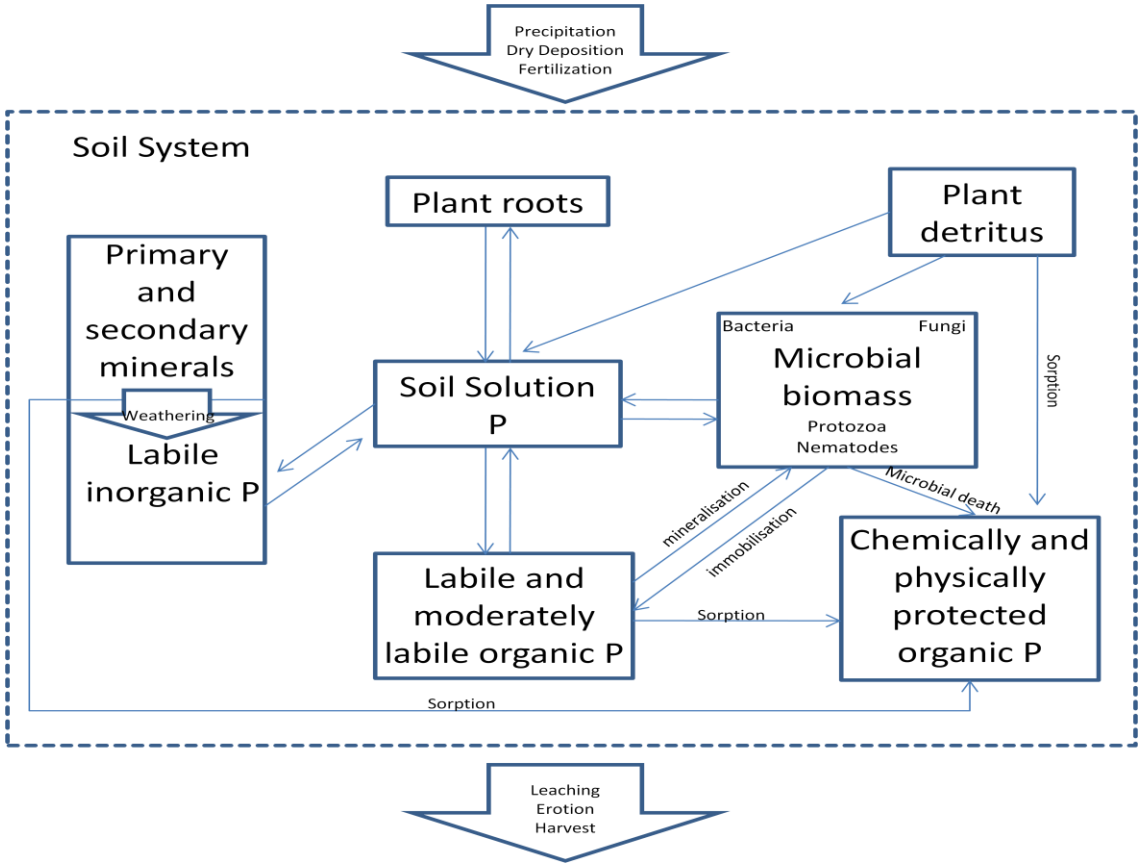

Figure 2.1 Soil phosphorus cycle.

Phosphorus bioavailability can be defined as the concentration of P in soil solution which is readily available for the plant and microbial demands. Soil solution is the main source of P for plants and microbes, and the majority of it is present as inorganic orthophosphates ($H_2PO^-_4$ or HPO^{2-}_4). However, when it is depleted by plant and microbial consumption it has to be replenished from a combination of inorganic, organic and microbial pools (Condron and Tiessen, 2005; Pierzynski et al., 2005).

Numerous studies have investigated the mechanisms which determine P bioavailability using different approaches such as P fractionation, enzyme activity, enzyme lability and ^{31}P nuclear magnetic resonance (NMR) spectroscopy. From these studies we know that the bioavailability of soil P is affected differently by the land use management, the soil physical and chemical characteristics, the type of vegetation cover and the microbial communities within it. For example, Chen et al. (2000) measured the soil phosphatase activity in soils under a 19 year old mixed forest stand of *Pinus ponderosa* and *Pinus nigra*, and an adjacent unimproved

grassland, and they found that the enzyme activity was higher in the topsoil under grassland compared to the forest. They concluded that the lower phosphatase activity observed under forest was related to corresponding lower biomass and microbial activities in the forest soil. Similarly, Turner et al. (2005), used ^{31}P NMR to quantify *scyllo*-inositol hexa*kis*phosphate in pasture soils to assess its potential bioavailability to growing plants, and they found that *scyllo*-inositol hexa*kis*phosphates represent a large and potentially bioavailable component of organic P in temperate soils.

The availability of inorganic P is generally high in young soils, and this relative abundance allows an enrichment of P in organic matter, during the progressive stages of ecosystem development (Allison et al., 2007; Condron and Tiessen, 2005; Turner et al., 2007b). However, as soil development continues the availability of P decreases due to several factors including organic immobilization, leaching, erosion and the transformation of inorganic P into occluded forms, mainly because of sorption and associations with iron and aluminium oxides and hydroxides (Figure 2.2) (Condron and Tiessen, 2005; Pierzynski et al., 2005; Turner et al., 2007b). Sposito (1986) explained that sorption and desorption reactions equilibrate with the soil solution because orthophosphates can be adsorbed to the surfaces and edges of hydrous oxides, clay minerals, and carbonates by replacing H_2O or OH^-. The sorption process involves two steps: 1) adsorption which is the accumulation of P on the surfaces of solid soil constituents; and 2) diffusion of P into solid constituents (Pierzynski et al., 2005; Sposito, 1986). Crop removal is also another major route by which P concentrations are lowered in the soil system (Pierzynski et al., 2005). Simpson et al. (2012) studied the relative solubility of soil P under contrasting mowing regimes. They found that P uptake was 51-54% higher under clipping-left treatments compared with clipping-removed and no mowing treatments. This suggested that biological and biochemical processes associated with enhanced mineralisation of organic P and turnover of P through the microbial biomass made a greater contribution to plant P uptake in the clipping left soils compared to other treatments.

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Figure 2.2 Changes in soil P content and forms with time (Walker and Syers, 1976).

All these factors result in an impoverishment of P in the organic matter pool and result in less accumulation in soils, affecting carbon (C) and nitrogen (N) cycling (Condron and Tiessen, 2005; Condron et al., 2005). In undisturbed ecosystems where the labile inorganic P fraction is small and the P cycle tightly closed, most of the P available to plants is supplied from the slow recycling of plant residue P through microbial processes in the soil (Richardson et al., 2005). On the other hand, McDowell and Condron (2012) reported results obtained from a long-term field trial in New Zealand which showed that soil organic P accumulated quickly as a consequence of superphosphate inputs. Similarly, Condron and Goh (1989a) found that the amounts and forms of organic P accumulated in an intensively grazed irrigated grassland after 25 years of phosphatic fertilizer application, although the rate of accumulation of organic P decreased with time due to increased mineralization as a result of lime addition.

As Bünemann and Condron (2007) explain, plant uptake and microbial immobilisation determine P fluxes in the soil, because otherwise the chemical equilibrium between different forms of P would only be affected by abiotic factors like moisture, temperature, atmospheric inputs and leaching losses. Interactions of soluble P with cations, the slow rate of P diffusion and conversion to organic forms, have promoted many adaptive strategies of plants to survive under P deficient conditions, some of which include establishing plant- microbe associations (mycorrhizae) and interactions with other non-symbiotic rhizosphere

microorganisms. Some of the most dramatic changes in plant roots are observed under inorganic P deficiency conditions leading to differences in root morphology and architecture, root hair density and length, nutrient absorption rate and the ability to modify the rhizosphere by releasing organic acids, enzymes and other substances (Chen et al., 2008; Raghotama, 2005; Vance et al., 2003). For example, Chen et al. (2003) in a glasshouse experiment found that radiata pine (*Pinus radiata*) produced more root exudates than ryegrass (*Lolium perenne*), which may have enhanced the dissolution of iron, aluminium and calcium minerals which in turn increased P mineralization.

Soil microorganisms comprise an important source and sink for nutrients like P, as well as being the main agents for transforming these elements (Unger et al., 2012) . Beever and Burns (1980) measured the efficiencies of inorganic P uptake from soil by microorganisms and plant roots using *in-vitro* experiments and observed that microorganisms, especially bacteria, had more efficient uptake mechanisms than plants. The soil fauna is less important as a P pool but promotes the turnover of the microbial biomass by grazing and predation (Bünemann and Condron, 2007). The equilibrium concentration of P in the soil solution, and the capacity of the soil to maintain this concentration against uptake by plant roots and microorganisms, is controlled by complex physico-chemical and biochemical mechanisms (Chen et al., 2008). The turnover of organic P is determined by immobilization and mineralization processes, where immobilization is the conversion of inorganic to organic P and mineralization is the process by which inorganic P is released from organic P in the soil (Condron and Tiessen, 2005; McGill and Cole, 1981).

Since P in the soil solution is the principal source of P for microbial uptake, microbial immobilization of P can be inferred from a decrease in phosphate in the soil solution and an increase in microbial biomass P (Bünemann and Condron, 2007). After microbial death, P immobilized can be re-mineralized or incorporated into soil organic P (Bünemann and Condron, 2007). With increasing time and weathering intensity, P loss from a system could be significant and a greater demand on organic P can slow its accumulation due to a decreasing contribution from inorganic P

(Richardson et al., 2004; Walker and Syers, 1976). McGill and Cole (1981) concluded that under such conditions the demand for internal cycling of organic P meets the needs for P increases, and suggest that hydrolysis of organic P would then be expected to increase, slowing its accumulation.

According to Chen et al. (2008), the fate and regulation of the phosphatase enzymes released by plant roots and microorganisms is complex, because they can be subject to adsorption, inhibition, biodegradation, stabilization and humification depending on the soil properties, and the type of predominant vegetation in the system. A study carried out on the Franz Josef chronosequence in New Zealand showed that the activities of phosphomonoesterase and phosphodiesterase enzymes increased with soil age, while their activity was negatively correlated with total P in soils (Allison et al., 2007).

2.2 Chemistry and Dynamics of Soil Organic P

2.2.1 Chemistry

Between 20 to 80% of the total P found in soils is made up of a combination of organic and microbial P, indicating that organic P is continually added to soil in plant, animal, and microbial residues (Bünemann and Condron, 2007; Condron et al., 2005; Rubaek and Sibbesen, 1994). Organic P represents between 30 to 60% of the total P in plants and up to 90% in soil microorganisms (Condron and Tiessen, 2005; Condron et al., 2005; Rubaek and Sibbesen, 1994; Turner et al., 2007a). Plants and microorganisms uptake inorganic P, which is bound to C moieties through phosphorylation by many biochemical processes (Condron et al., 2005). According with Condron and Tiessen (2005), organic P is defined as P bonded with C (commonly via ester bonds (C-O-P)), which originates from a combination of animal, plant and microbial residues.

Organic P compounds are classified into orthophosphate esters, phosphonates and orthophosphate anhydrides based on the nature of the P bond (Figure 2.3) (Condron *et al*., 2005). Tate (1984) explained that the chemical diversity of soil organic P is

wide due to organic moieties. Orthophosphate esters are subdivided into monoesters and diesters depending on the number of ester groups linked to each orthophosphate ion.

Orthophosphate monoesters are the most common form of organic P in the majority of soils and can represent up to 100% of total soil organic P (Condron et al., 2005). These include sugar phosphates, phospho-proteins, mononucleotides and inositol phosphates, the last group being the predominant form of orthophosphate monoesters found in most soils (Celi and Barberis, 2005; Condron et al., 2005). Inositol hexakis and pentakisphosphate are the most abundant forms of inositol phosphates present in soil and are believed to be derived primarily from plants. These forms comprise up to 60% of identified soil organic P together with much smaller amounts of nucleotides, sugar phosphates and phosphoproteins, while a large proportion of the remaining unidentified organic P which may be insoluble complexes with clay minerals and organic matter. Orthophosphate diesters are found in nucleic acids, phospholipids, teichoic acids and aromatic compounds, all of which occur in much smaller concentrations compared with those of orthophosphate monoesters (Condron and Tiessen, 2005; Condron et al., 2005). Nevertheless, these are considered to be the most bioavailable forms of organic P and are the predominant species found in plant and microbe cells (Quiquampoix and Mousain, 2005).

Sorption of P onto soil minerals is strong and is more evident for certain organic P compounds, like inositol hexakisphosphate, which includes six phosphate groups, three of which can get adsorbed onto iron and aluminium oxides-hydroxides and clays and consequently decreases its availability to enzymes and microorganisms (Bünemann and Condron, 2007). Thus inositol phosphates are generally considered to be recalcitrant compared with diester forms of organic P.

Turner et al. (2007b) observed that the proportion of inositol phosphates declined markedly during the late stages of a 120,000 Franz Josef post-glacial chronosequence in New Zealand. On the other hand, the proportions of diester DNA increased

continually throughout the sequence. One explanation is that the decline in inositol phosphates could be related to the weathering of amorphous metal oxides to more crystalline forms which increase the bioavailability of inositol phosphates, while DNA may be stabilized by incorporation into organic matter structures.

Condron et al. (2005) concluded that despite advances in the identification of the chemical forms of organic P, several factors make its identification difficult, such as the chemical complexity of soil organic P, the susceptibility of some organic P compounds to hydrolysis during extraction, strong sorption of organic P by clays and the formation of insoluble salts with metal cations.

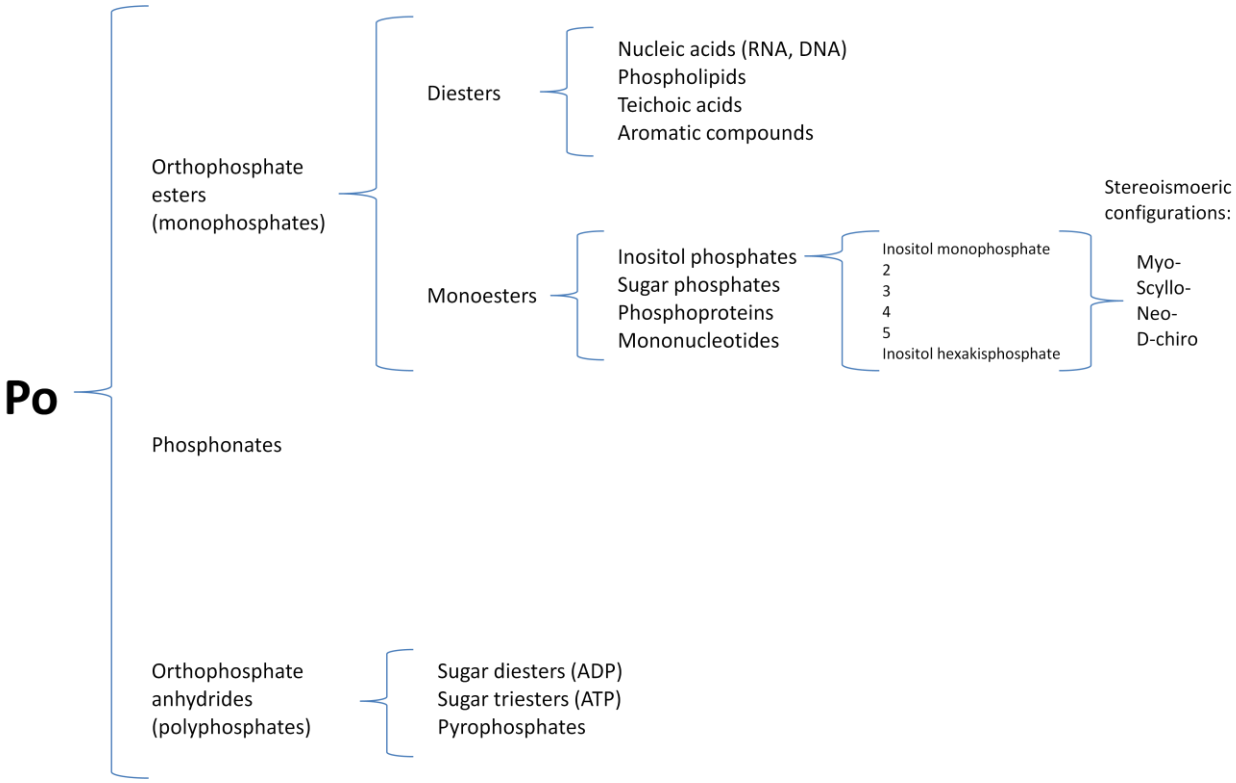

Figure 2.3 Soil organic P (P_o) classification.

2.2.2 Dynamics

In both natural and agricultural systems the incorporation of plant, animal and microbial residues (above- and below-ground) into soil provides the energy needed to sustain nutrient cycles, as well as ensuring that the P they contain re-enters the P cycle (Turner et al., 2007a). McGill and Cole (1981) conducted an extensive review of nutrient mineralization in soil and concluded that while carbon, nitrogen and some

sulphur forms are mineralized due to microbial oxidation of organic carbon to provide energy (biological mineralization), organic P and other forms of sulphur are mineralized by the action of extracellular enzymes like phosphatase and sulphatase (biochemical mineralization), which are produced by plant roots, mycorrhizae and microorganisms in the soil in response to low nutrient availability (Bünemann and Condron, 2007; Prosser, 2002).

Biochemical mineralization is defined as the release of inorganic P ions from organic matter components due to decomposition of plant and microbial detritus through enzymatic catalysis (hydrolysis by phosphatases) external to the cell membrane. The process is strongly controlled by the need for the element released rather than the need for energy (Bünemann and Condron, 2007; Condron and Tiessen, 2005; McGill and Cole, 1981). Therefore a bell-shape curve is evident with respect to C:Po and N:Po ratios in climosequences from immature aggrading systems through the climax with respect to C content and finally to degrading systems, indicating that P limitation in the late stages of ecosystems can cause a decline in forest biomass, productivity, plant community composition and lower species diversity (Turner et al., 2007b; Walker and Syers, 1976; Wardle et al., 2004). However, some studies indicate that dynamics of organic P and C are more closely related than the model suggested by McGill and Cole because C is important for the early patterns of mineralization processes as the turnover of C in a system increases through plant addition (plant residues release P very fast since most of it is found as inorganic P). Thus, the biological processes of mineralization determine the availability of P (Bünemann and Condron, 2007).

Microbial uptake of P can be less than, equal to or greater than organic P mineralization and consequently P in the soil solution can be replenished or depleted by microbial turnover, affecting the availability of P to plants (Oberson and Joner, 2005). Oberson and Joner (2005) defined net mineralization as the difference between gross mineralization and immobilization, while re-mineralization is the release of microbial P after the decomposition of organic materials due to microbial death or predation. Microbial P compounds are affected by the composition of the

microbial community, the P content on the medium, and cell age which in turn is affected by the living conditions of the soil microorganisms. Some studies conducted in New Zealand revealed that microbial P constitutes between 0.5% of the total P in grassland soils and 26% of the total P in the litter layer of a podocarp forest (Chen et al., 2000; Ross et al., 1999). In mineral topsoils, microbial P increases in the following order: arable < forest < grassland (Oberson and Joner, 2005). The turnover of microbial P has two components: 1) temporal net changes in microbial P, and 2) microbial activity. The turnover does not necessarily manifest a change in net pool size and consequently the rate of turnover regulated by net changes can be estimated from the fluctuations in the microbial P pool.

An increase in microbial P indicates immobilization in microorganisms, while a decrease indicates that P has been released from them (i.e. mineralized). Seasonal fluctuations in microbial P concentrations are negatively correlated with available P (Oberson and Joner, 2005). For example, Oberson et al. (1999) found an increase in microbial P related to a decrease in available P in an unimproved tropical pasture in Colombia. Temporal fluctuations in microbial P have been used to estimate the annual P flux through the soil microbial biomass and the turnover time of microbial P under field conditions. Chen et al. (2003) estimated the annual P release from the soil microbial biomass to be approximately 30 mg P/kg/yr under grassland and 35 mg P/kg/yr under nearby forest. The turnover rate was higher in the forest soil than in the grassland, resulting in turnover times of 0.8 and 1.3 years for soil microbial biomass in forest and grassland soils, respectively.

Soil organic P mineralization cannot be accurately estimated from changes in extractable Pi, because some of the mineralized P will be rapidly adsorbed on soil colloids. According to Oberson and Joner (2005) estimates of organic P mineralization are essentially based on two approaches: 1) comparison of native and cultivated soils at sites with similar history, and 2) determination of gross P mineralization rates by isotopic techniques. Phosphorus immobilization by microorganisms, turnover of microbial phosphorus and mineralization of microbial by-products, are the major processes regulating phosphorus cycling and availability

from organic material, for example, organic P mineralization in the litter layer is generally recognized as the major process providing available phosphate in forest (Attiwill and Adams, 1993; Vitousek et al., 2010). Microorganisms use a wide variety of mechanisms that solubilize or desorb solid-state P and acquire P from soil solution, including release of extracellular enzymes, liberation of mineral dissolving ions or molecules, cellular membrane transport involving specific permeases or passive diffusion through the membrane lipidic bilayer, and active secretion of low molecular weight organic acids that can solubilize precipitated phosphates by decreasing pH in their vicinity (Allison et al., 2007; Chen et al., 2000; Chen et al., 2008; George et al., 2009; Oberson and Joner, 2005; Quiquampoix and Mousain, 2005; Richardson, 2001). All organic P forms and some inorganic P forms can be hydrolyzed by a class of enzymes broadly defined as phosphohydrolases which are proteins that can catalyze the transfer of P from P_{enzyme} to $P_{membrane}$, by allowing the reduction of polymers (most of the organic matter is found as polymerized forms) into smaller molecules that are more soluble and can diffuse in the soil pore network (Quiquampoix, 2000; Quiquampoix and Mousain, 2005). Phosphohydrolases are a class of the phosphomonoesterases which can be subdivided into acid and alkaline phosphatases depending on the optimum pH they need to hydrolyze, while phytases are phosphohydrolases which can degrade *myo*- inositol hexakisphosphate (Quiquampoix and Mousain, 2005). The activity of phosphatase enzymes in soil is also influenced by the amounts and bioavailability of metals such as magnesium and cobalt (Quiquampoix and Mousain, 2005; Turner et al., 2002).

2.3 Soil Phosphorus Dynamics in Grasslands and Forest Systems

2.3.1 Grassland Systems

As Kemp et al. (2000) indicated, grazed pastures are open ecosystems that comprise animals, plants (pasture) and soil, which determine soil P transformation. Nutrient cycling in pastures includes atmospheric inputs, inputs in the form of fertilisers and outputs via gaseous emissions, leaching, surface runoff and in off-farm products (Kemp et al., 2000; McLaren and Cameron, 1996). Kemp et al. (2000) explained that on an annual basis, most of the nutrients taken up by plants are returned to the soil

in the form of litter and root residues (10-70%) or animal excreta (50-95%). Figure 2.4 shows the components and major pathways associated with nutrient cycling in grazed pasture

Pasture production in New Zealand is influenced by the application of fertilisers because soils are deficient in N, P and sulphur. The nutrient requirements of pasture species vary, resulting in competition between species under soils with different fertility levels. Legumes require more P than grasses and as most of the N input into pastoral systems comes from N fixation via legumes, it is critical to apply P fertiliser to maintain or increase the soil P level if an effective proportion of legume species is expected to be maintained in the pasture (Kemp et al., 2000).

The variability in pasture botanical composition is also dependent within field soil fertility gradients. One of the most important factors causing fertility gradients is the nutrient transfer arising from uneven deposition of dung and urine by grazing animals, because the addition of nutrients in urine and dung will substantially increase pasture growth and nutrient content compared with adjacent untreated areas (Kemp et al., 2000). For example, a study conducted by Matthew et al. (1994) showed that ryegrass/clover (*Lolium perenne*/*Trifolium repens*) associations dominated at microsites with higher levels of soil P due to urine and dung deposition, while other species like sweet vernal (*Anthoxanthum odoratum*) replaced ryegrass at medium fertility microsites and Yorkshire fog (*Holcus lanatus*) dominated at lower fertility microsites.

Pasture composition also changes within time in response to soil fertility status. It has been observed that grasses like browntop dominate at the early stages of pasture development when soil fertility is relatively low, but increasing soil fertility favors the dominance of ryegrass and other high fertility grasses (McLaren and Cameron, 1996).

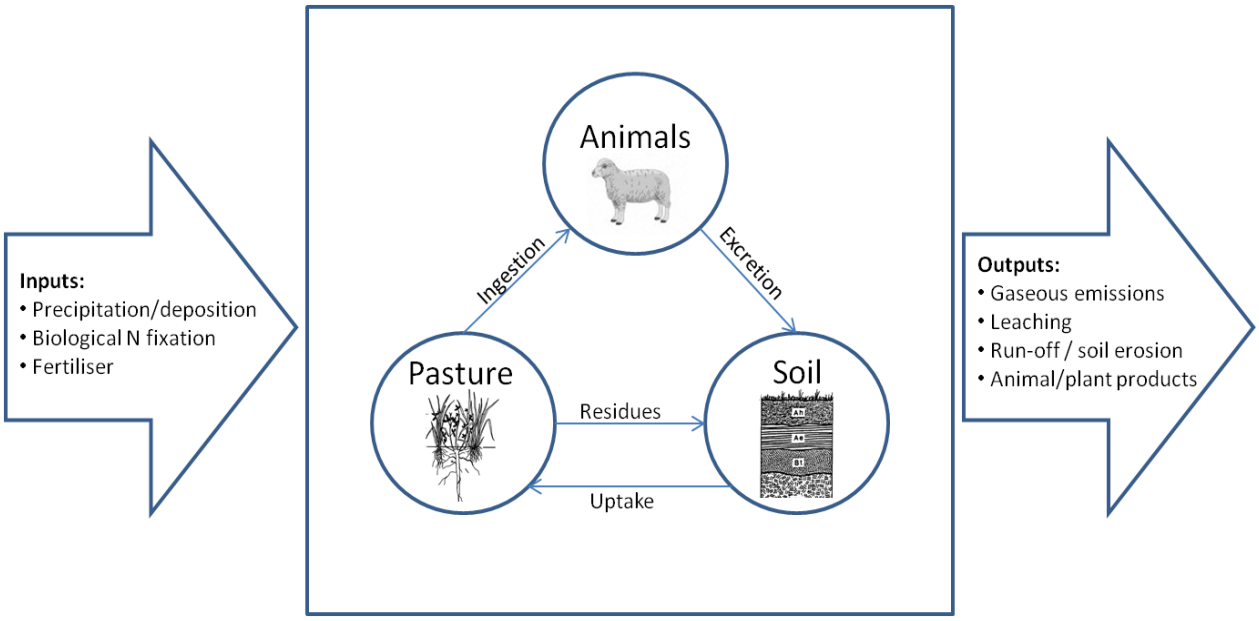

Figure 2.4 Nutrient cycling in pastures, showing the key pools and processes (Kemp et al., 2000) .

2.3.2 Forest Systems

A characteristic of forest ecosystems is the development of a distinct forest floor resulting from the periodic returns of litter, branches, bark and fruit, which contain a variable proportion of the nutrients extracted annually by the trees from the soil (Condron, 2006; Vitousek et al., 2010). The litter on the forest floor decomposes, liberating nutrients which can be immobilized or mineralized (Figure 2.4) (Condron, 2006; Pritchett and Fisher, 1987; Vitousek et al., 2010). Chapin (1980) suggested that the nutrient use efficiency of a forest is inversely proportional to the nutrient concentration in the aboveground litterfall, root turnover, and the organic matter increment of the vegetation (which in an older forest is mostly wood). According to Vitousek et al. (2010) organic matter, N, P and calcium move largely in litter fall and consequently the efficiency of these nutrients for use in aboveground litter production, in a wide range of sites, can be calculated from existing data. Chen et al. (2008) conducted an extensive review of organic matter and P cycling in different forest ecosystems and concluded that the amount of dry matter produced per unit of P is systematically lower in temperate compared to tropical forests. Kavvadias et al. (2001) observed that the low nutrient concentrations of P in the foliage of *Pinus pinaster* was reflected in the chemical composition of litter (94 ± 0.435 mg P/g) and P

concentration of the forest floor (0.56 ± 0.097 t P/ha) in a temperate forest in Greece.

Differences in organic matter deposition and nutrient cycling between forestry plantations and other types of managed ecosystems have a significant impact at ecosystem level, because they affect the mineralization and immobilization processes (Figure 2.5). Fisher and Stone (1969) were some of the first to report that mineralization of organic P was higher under pine species compared with adjacent abandoned fields and larch plantations, indicated by an increase in the concentration of available inorganic P and low levels of total and organic P in the root zone of pines. In addition, studies conducted in New Zealand have demonstrated that despite the increased mineralization of organic P under recently established forest compared with adjacent grasslands, levels of microbial biomass P were lower under forest as well as the activities of extracellular enzymes responsible for organic P mineralization (Chen et al., 2008; Condron et al., 2005; Davis, 1994). Chen et al. (2008) concluded that decreases in soil microbial biomass and activity under forest may be attributed to reduced inputs of labile organic matter in addition to decreases in soil pH. Davis (1994) conducted an experiment to compare the properties of topsoils collected under a plantation of *Pinus radiata* and an adjacent *Chionochloa rigida* grassland, and found that moisture content, pH and concentrations of total and organic P were lower under the *P. radiata* stand compared to the grassland, which he attributed to increase nutrient uptake and mineralisation of organic matter by the pines. Chiu et al. (2005) reported that plant-available inorganic P was greater in soils under Chinese hemlock (*Tsuga chinensis*) compared to dwarf bamboo (*Yushania niitakayamensis*) and alpine silver grass (*Miscanthus transmorrisonensis*), while an NMR analysis indicated that inorganic orthophosphate and orthophosphate monoesters were the major forms of P extracted in forest and grasslands, respectively. In addition, some studies have shown that decreases in orthophosphate monoesters and *myo* and *scyllo* inositol hexakisphosphate in soils under pine indicated the utilization of such compounds by the coniferous plants through root-microbe associations (Chen et al., 2008; Chiu et al., 2005; Turner et al., 2007b).

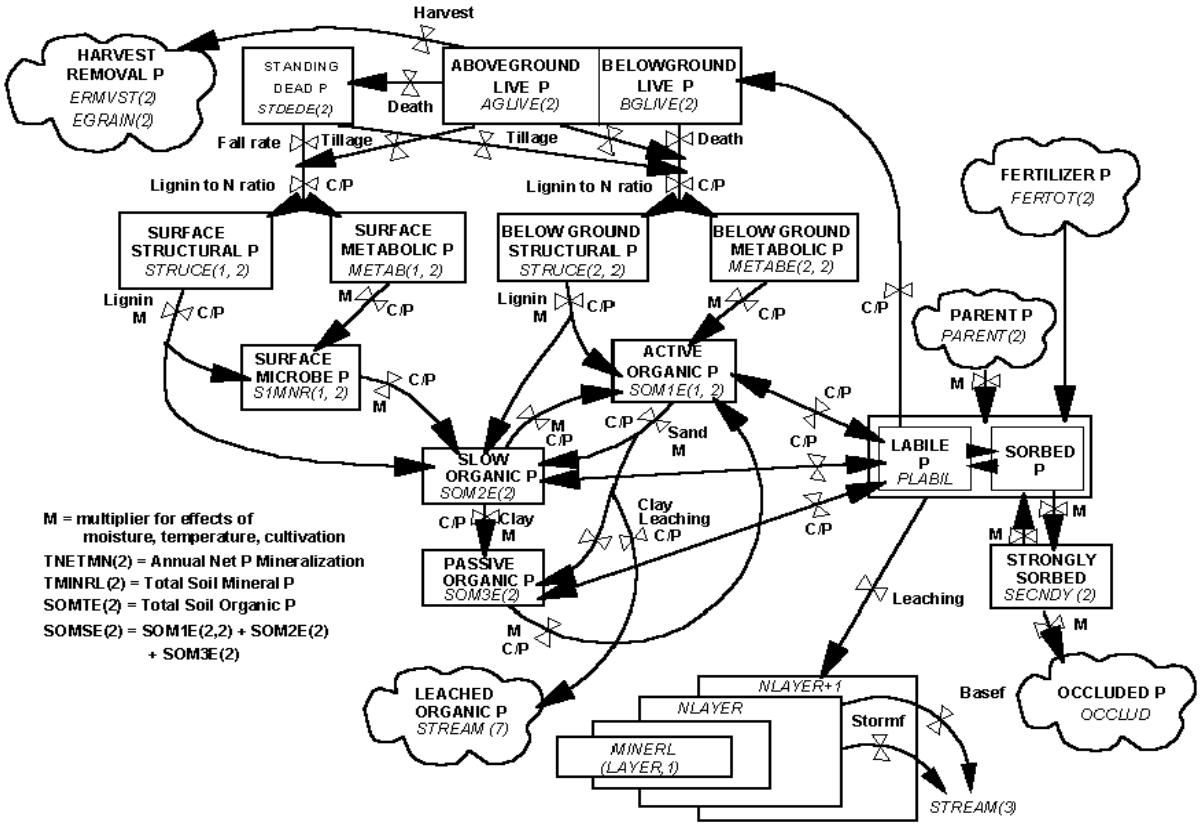

Figure 2.5 Phosphorus dynamics in forest and cultivated ecosystems (Metherell et al., 1993).

Silvopasture or pastoral agroforestry refers to the combination of conifer species and grazed pasture and has been consider a viable productive system (Knowles, 1991). According to Mead (1995), the most common silvopastoral species grown in New Zealand are *P. radiata*, ryegrass (*Lolium perenne*) and white clover (*trifolium repens*). Some studies have revealed that these species show differences in the utilization of soil inorganic and organic P (Davis, 1995; Mead, 1995; Scott and Condron, 2003). For example, Davis (1995) observed that *P. radiata* mineralized more organic P and increase the availability of inorganic P than cocksfoot (*Dactylus glomerata*), indicating that different understorey species grown in silvopastoral systems can affect the forms and availability of soil P.

Other studies have shown that plant root-microbial interactions in the rhizosphere are very important in the transformation of soil organic C and associated nutrients because plant roots secrete various chemical compounds like sugars, amino acids, organic acids, hormones, vitamins and enzymes into the soil (Chen et al., 2008;

Kourtev et al., 2003; Niu et al., 2007). Such exudates are considered to act as a signal to initiate and manipulate biological and physical interactions between roots and soil organisms (Bünemann and Condron, 2007; Kourtev et al., 2003). Chen et al. (2008) explained that plant species stimulate growth of different microbial communities in the rhizosphere, possibly due to differences in root exudation, so plants can be considered the major drivers of microbial activity, community structure and function.

Some soil microorganisms can modify plant root structure and function via mycorrhizal associations (Colpaert and vanTichelen, 1996; Conn and Dighton, 2000; Jakobsen et al., 2005). These mycorrhizal associations are critical to mediating the availability of soil P to many plants. Vesicular-arbuscular mycorrhizae (VAM) are generally associated with grasslands and some woody species, while ectomycorrhizae (ECM) are found in a limited number of families, including Pinaceae, Betulaceae, Fagaceae, and Myrtaceae (eucalyptus) (Chen et al., 2008; Jakobsen et al., 2005). In general, ECM tend to be more efficient than VAM in P uptake and transportation to the host because they can degrade cellulose in sterile conditions and phosphohydrolase production is activated even in the absence of P (Chen et al., 2008). For example, Colpaert and vanTichelen (1996) conducted a greenhouse experiment and observed that P concentrations in leaves of Scots pine (*Pinus sylvestris* L.) were higher in plants inoculated with ECM compared with plants without the treatment.

Moreover, some studies have provided evidence that ECM release significant quantities of low molecular weight organic acids which promote solubilisation of recalcitrant forms of organic P and its consequent mineralization (Chen et al., 2003; Chen et al., 2008; Cieslinski et al., 1998). Another study conducted by Niu et al. (2007) found that an exotic understory species (*Ageratina adenophora*) increased the abundance of VAM, the fungi/bacteria ratio and the levels of available P, potassium, ammonium N and nitrate, suggesting significant influences between roots and mycorrhizal associations.

Root exudates containing low molecular weight organic acids such as citric, oxalic, maleic, and acetic acids, can also increase the availability of soluble Pi minerals by a combination of lower pH, metal chelation and oxidation-reduction reactions in the rhizosphere and thus increase their susceptibility to microbial attack and enzyme hydrolysis (Chen et al., 2008; Cieslinski et al., 1998). Chen et al. (2008) concluded that the rate of mineralization was higher under pine forest compared to an adjacent grassland, suggesting that pine roots and their association with ectomycorrhizal fungi release organic acids which dissolved aluminum- and iron-organic P complexes by chelation and consequently liberated organic P. They also proposed that organic acids acidify the rhizosphere and could also promote the solubilisation of inorganic and organic P.

Since P mineralization supplies a significant proportion of the total P requirements for plants, changes in soil organic P are correlated to changes in plant and microbial communities, suggesting that the distribution of species depends on the ability to access soil organic P (Oberson and Joner, 2005; Turner et al., 2007b). For example, Kourtev et al. (2003) found that changes in enzyme efficiency and soil nutrient concentrations were accompanied by shifts in the composition of the microbial community in a temperate forest. In addition, Turner et al. (2007b) observed a shift between evergreen angiosperm forests on young soils to conifer forests on older soils, which was accompanied by an increase in fungi compared with bacteria in soil. It has also been recognized that plant uptake and microbial immobilization have a great potential to disrupt the equilibrium of inorganic nutrients (Bünemann and Condron, 2007; Yeates et al., 1997). Several studies have demonstrated that there is depletion of soluble organic P during plant growth, indicating a contribution of organic P to plant nutrition through mineralization (Chiu et al., 2005; Condron et al., 2005; Vance et al., 2003; Yeates et al., 1997).

Studies conducted in temperate forest showed that seasonal changes in environmental conditions (rainfall, soil moisture and temperature) influence the biological and biochemical processes involved in P cycling (Chen et al., 2008; Condron et al., 2005; Gburek et al., 2005; Tiessen, 2005). Chen et al. (2003) found

that mineralization of labile organic P during spring and summer was higher because microbial activity responded to an increasing plant demand in those seasons, while during autumn and winter there was an accumulation of organic P as a result of increased organic inputs, slower plant growth and low microbial activity in a temperate forest of New Zealand. Similarly, Scott and Condron (2003) and Chen et al. (2003) found lower levels of labile organic P and higher inorganic P in tree and forage rhizosphere in spring compared to autumn in a temperate agroforestry system.

Temperature, moisture, aeration, soil reaction and the history and intensity of land management are all factors that control the population dynamics, distribution and activities of soil microorganisms and plants in temperate forest and consequently the balance between mineralization and immobilization of P in soil (Condron et al., 2005; Scott and Condron, 2003). Land-use change has been proved to be a major factor that influences organic P mineralization. Condron et al. (2005) explain that transformations of organic P under land-use change are closely linked to reductions in soil organic matter, resulting in a net mineralization of soil organic matter. They indicate that P is mineralized as a consequence of overall organic matter mineralization, causing a decrease in soil organic C and P. Afforestation of grassland with conifers promotes mineralization of soil organic matter and associated P, increasing the levels of available inorganic P in soils. This can be due to a combination of factors such a greater demand and uptake of P by trees, improved solubility of organic P by root and microbial exudates, more phosphatase activity associated with ectomycorrhizae (association favored by conifers), together with changes in soil moisture and temperature. It is clear from the above that land use management has a significant impact on the nature, distribution and dynamics of soil P (Chen et al., 2008; Chiu et al., 2005).

CHAPTER 3 RESEARCH HYPOTHESES AND OBJECTIVES

From numerous pair site studies we know that the afforestation of grassland has profound effects on the nature and bioavailability of soil P, resulting in enhanced mineralization of organic P in particular. However, most of these studies were conducted at a particular point of the time following forest establishment (typically 10-20 years), and consequently little is known about the timing and nature of changes in soil P cycling that occur as a result of afforestation. Accordingly, more detailed research is justified to elucidate temporal changes in soil P associated with afforestation, as well as the effect of different plantation forest tree species on the amounts, forms and bioavailability of soil P. To address this, a suite of four hypotheses was proposed for the current study to investigate the effects of time and tree species on soil P dynamics following grassland afforestation and in relation to forest ecosystem development. This approach is outlined below.

HYPOTHESIS	OBJECTIVE	CONTEXT
Seasonal variations in the quantities and nature of soil P would be influenced by different tree species.	To investigate seasonal changes in soil P under three tree species over a 12 month period.	Orton Bradley Park (OBP) field experiment (Chapter 4)
The amounts and forms of soil P would change within time after forest establishment of different plant species.	To investigate and quantify changes in soil P that occurred over 5 to 28 years after the establishment of trees on grassland.	OBP field experiment (Chapter 4) Glendhu field experiment (Chapter 5) Lincoln University Silvopastoral Field Trial (Chapter 6)
Tree species would have a significant impact in the nature and availability of soil P as a consequence of different growth and P acquisition strategies.	To determine the effects of different tree species on the chemical nature, dynamics and bioavailability of soil P.	OBP field experiment (Chapter4)
The bioavailability of soil P in a dune chronosequence will decline within time, with rapid depletion of primary mineral P and the accumulation of organic P.	To quantify changes in soil P along a chronosequence, and assess its bioavailability.	Haast chronosequence (Chapter 7)

CHAPTER 4 CHANGES IN SOIL PHOSPHORUS WITH TIME DURING THE EARLY STAGES OF AFFORESTATION OF GRAZED HILL COUNTRY PASTURE

4.1 Introduction

Most previous research focusing on the impacts of grassland afforestation on soil P dynamics and availability is limited as it has been based on paired site studies at a single point in time, commonly 10 to 20 years after forest establishment (Chen et al., 2000; Chen et al., 2008; Condron et al., 1996; Davis and Lang, 1991). Details of the processes responsible for the observed changes in soil P occurring as a consequence of afforestation are therefore unavailable. Other studies have revealed that different tree species can promote changes in the quantity and quality of organic inputs such as leaf litter and root turnover, and this can modify the biomass and diversity of soil microbial communities, which in turn, affects soil P dynamics (Kourtev et al., 2003; Niu et al., 2007). A closer examination of the changes in soil P over time following the establishment of different tree species will improve our understanding of the mechanisms responsible for changes in P dynamics and availability.

The Orton Bradley Park field trial was established in 1999 to facilitate the investigation and quantification of temporal changes in soil properties following afforestation of grazed ryegrass-clover pasture (endomycorrhizal) with three contrasting species, namely *P. radiata* (ectomycorrhizal), *C. macrocarpa* (endomycorrhizal) and *E. nitens* (ecto and endomycorrhizal).

The main objective of this study was to determine the effects of different tree species on the chemical nature, dynamics and bioavailability of soil P, using detailed analysis of soil samples collected at the time of forest establishment, and again 5 and then 10 years later. This was complemented with a seasonal investigation of changes in soil P under the three tree species over a 12 month period in 2011-12 (12-13 years after establishment). It was hypothesised that tree species would have a significant impact on temporal and seasonal differences in the nature and availability of soil P as a consequence of different growth and P acquisition strategies.

4.2 Orton Bradley Park Temporal Study

4.2.1 Materials and Methods

Orton Bradley Park is located near Charteris Bay on Banks Peninsula, Canterbury, New Zealand (43º 39´ S, 172º 42´ E). The soil is a Takahe silt loam (Mottled Fragic Pallic soil, NZ classification; Typic Fragiustept, USDA classification) formed in greywacke loess. The altitude is 100-150 m, mean annual rainfall is approximately 1000 mm and mean annual temperature is 12.1º C. A replicated afforestation trial was established by Lincoln University in 1999 at the site in a 2 ha area developed under grazed pasture with limited fertilizer inputs on the lower north-east aspect slopes (70-150 m elevation), with the aim of investigating and quantifying temporal changes in soil properties and processes following afforestation of grazed pasture with different tree species. The trial area consists of 4 replicates of 3 commercial plantation forest trees (*Pinus radiata*, *Eucalyptus nitens* and *Cupressus macrocarpa*) arranged in a randomized block design, in twelve plots each measuring 30 m x 30 m (900 m^2) (see Figures 4.2.1, 4.2.2 and 4.2.3). The plots were not grazed or fertilized since trial establishment and the trees have been pruned and thinned in accordance with commercial silviculture practice.

Figure 4.2.1 Satellite view of the field research site at Orton Bradley Park (source: Google Earth).

Figure 4.2.2 Field trial site at Orton Bradley Park in a) 2000, b) 2004 and c) 2009.

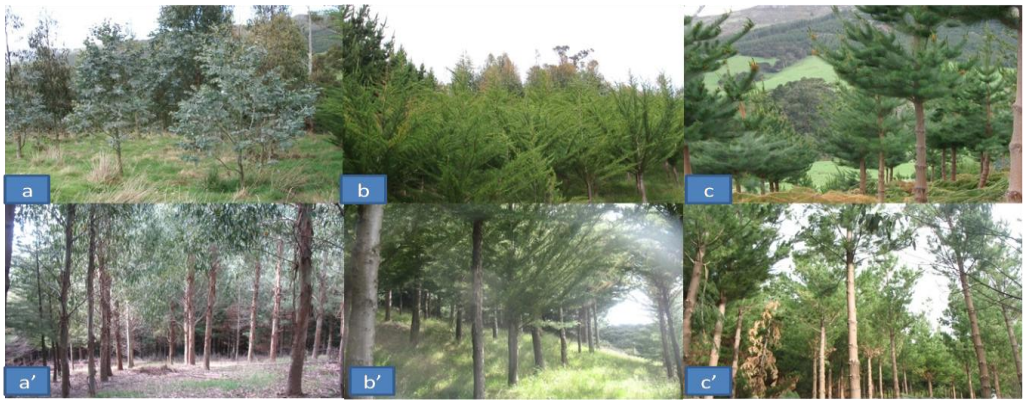

Figure 4.2.3 Field trial site at Orton Bradley Park. a) *Eucalyptus nitens* in 2004, a') *Eucalyptus nitens* in 2009, b) *Cupressus macrocarpa* in 2004, b') *Cupressus macrocarpa* in 2009, c) *Pinus radiata* in 2004 and c') *Pinus radiata* in 2009.

A detailed soil sampling protocol was developed for the trial at its inception in 1999. Samples of mineral soil were taken using an Ellenkamp corer (6 cm diameter) at 3 depths (0-5, 5-10, 10-20 cm) and bulked together from five sites randomly located in the middle of each replicate plot at three points in time: September 1999 (at trial establishment), September 2004 (5 years after planting) and November 2009 (10 years after planting). Soil samples were collected from each of the 4 replicate plots of each species (12 plots), air-dried at 30 ºC for five days, sieved <2 mm and stored in plastic containers prior to soil analysis.

4.2.1.1 Soil Analysis

Soil P fractionation

Available P is a functional concept rather than a measurable quantity and consequently, no simple direct measure is available (Tiessen and Moir, 1993). A variety of techniques have been developed to examine different forms of P in soil, generally involving extraction with specific reagents to recover particular P compounds (Condron *et al*., 2005). As Condron *et al*. (2005) explain, sequential fractionation methods were developed to measure soil P with respect to its chemical bonding, which involve exposing soil samples to increasingly stronger extractants to separate soil P into fractions based on soil P solubility in a range of neutral, alkaline and acid reagents.

These fractionation methods have the advantages of only requiring small quantities of soil, are relatively simple to perform, and require only basic lab equipment, but on the other hand, the strongly alkaline solutions can promote the hydrolysis of organic P, as well as the readsorption-precipitation of some inorganic P compounds (Barbanti et al., 1994; Condron et al., 2005). The step to remove organic matter from extracts may also remove inorganic P linked to humic substances, and consequently overestimate organic P (Gerke, 1992). Another important disadvantage is that various extracts are not specific for any particular group of organic P (Chen *et al*., 2002; Condron *et al*., 2005), and therefore, information about organic P compounds obtained by sequential fractionation should be taken with caution and must be coupled with other more reliable techniques such as enzyme labile P or ^{31}P NMR .

A method developed by Hedley *et al.* (1982) was aimed to quantify labile inorganic P, Ca-associated Pi, Fe+Al associated inorganic P, in addition to labile and more recalcitrant forms of organic P. Labile forms of inorganic P were extracted with resin and bicarbonate, while recalcitrant forms of organic P and less available inorganic P were extracted using sodium hydroxide (NaOH) and hydrochloric acid (HCl) (Hedley *et al*., 1982). The original fractionation (Hedley *et al*., 1982) left between 20 and 60% of the P in the soil unextracted. This residue often contains significant amounts of

organic P that sometimes participate in the relatively short-term transformations. This residual organic P can be extracted using NaOH after the acid extraction (HCl).

For this study, the amounts and forms of inorganic and organic P in soils were determined using the sequential fractionation scheme outlined by Chen *et al*. (2000), which in turn was a modification of the method developed by Hedley *et al*. (1982) . This scheme involved sequential extraction of soil with 1 M NaCl, 0.5 M NaHCO3 (pH 8.5), 0.1 M NaOH (I), 1 M HCl and 0.1 M NaOH (II) , with details shown in Figure 2.2.4. Total extractable soil P was determined as the sum of extractable P fractions. In order to obtain the inorganic P of each fraction, 5 mL of extract were taken (just 1 mL for the fractions in NaOH) and placed in 25 mL flasks. Approximately 8 mL of deionized water was added, followed by 0.55 mL of 5 M HCl. Once the extract stopped effervescing, the sample was neutralized by adding 2 drops of P-nitrophenol, 0.4 mL of 10 M NaOH (0.81 mL for HCl extracts) and drop by drop of 5 M HCl until the solution turned clear. Finally, 4 mL of colour reagent (Murphey-Riley) was added, and it was made up to the volume of the flask with deionized water before the absorbance was measured after 12 minutes at 880 nm.

Concentration of organic P was calculated as the difference between total P and inorganic P. For determination of total P in the extracts, 5 mL of extract (only 1 mL for the fractions in NaOH) was added to digestion tubes, followed by 5 mL of 6 M H_2SO_4 and 0.7 mL of a 50% solution of $(NH_4)_2\ S_2O_8$ (ammonium peroxydisulfate). The tubes were then autoclaved at 121°C for 60 min. Extracts were neutralized by adding drops of 10 M NaOH and 5 M HCl, and made up to total volume (25 mL) after adding 4 mL of Murphy Riley colour reagent. After 10 min, the absorbance was measured at 880 nm. The concentrations of inorganic P in the NaCl extract as well as the inorganic P in the $NaHCO_3$ extract were added together to represent the most labile form of P (Labile P). Total extractable inorganic P and organic P were determined by summing P in the various component fractions. The soil samples were analyzed in batches correspondent to each year (1999, 2004 and 2010) with two replicates for each sample and two internal standards that were used in all the batches.

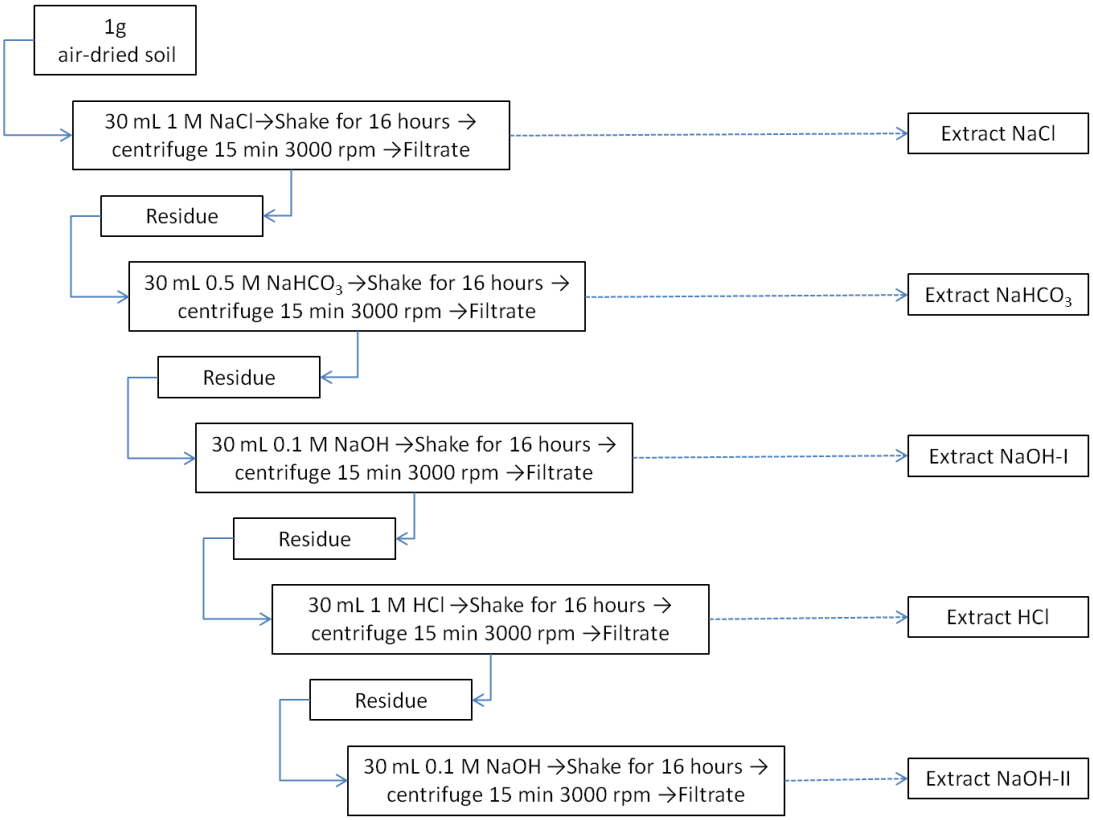

Figure 4.2.4 Soil P fractionation (Chen et al., 2000).

Enzyme- labile soil organic P

Characterization of organic P is based on the principle that substrate-specific phosphate enzymes will release inorganic P from specific organic P forms. Consequently, it is possible to group organic P forms by compound categories after adding commercially available phosphatase enzymes to soil extracts and colorimetrically analyzing the inorganic P released. The specific classification of P forms will depend on the type of enzymes used. Alkaline phosphatase will hydrolyze labile monoester P (Ortho-P), while phytase will hydrolyze just phytic acid (*myo*-inositol hexakisphosphate) (O'Halloran and Cade-Menun, 2008).

The method consisted of adding 30 mL of 0.5 M $NaHCO_3$ to 1.5 g of soil and shaking for 30 minutes (two blanks were included) followed by centrifuging at 2000 g for 15 minutes and filtering of the extract supernatant through Whatman No. 5 paper. One mL of extract was placed in 50 mL centrifuge tubes and acidified by adding 0.1 mL of 3 M H_2SO_4 and neutralized by adding 0.12 mL of 1 M NaOH. One mL of 25 mM sodium azide (NaN_3) was added to prevent microbial activity and 0.25 mL of each

enzyme (alkaline phosphatase from *Escherichia Coli* and phytase from *Aspergillus ficuum*; SIGMA Chemicals) was added to the appropriate labeled tube for each sample and blank. Control tubes were left enzyme free, but 0.25 mL of 2mM magnesium chloride ($MgCl_2$) was added. All the samples were diluted to 5 mL with deionized water and incubated at 37°C for 16 hours, and the enzyme reaction terminated by adding 1 mL of colour developing solution (Murphy-Riley B). The absorbance was then measured after 12 minutes at 880 nm. The inorganic P concentration in solution was calculated using a standard curve and all samples were duplicated. Concentrations of labile monoesters released by alkaline phosphatase and phytase enzymes were determined in 0-5 cm soil collected in 1999, 2004 and 2009 under Pinus *radiata* (4 replicates per year).

^{31}P Nuclear magnetic resonance spectroscopy (NMR)

Detailed analyses of organic P forms (inorganic orthophosphate, orthophosphate monoesters, orthophosphate diesters, pyrophosphate and phosphonates) using ^{31}P nuclear magnetic resonance spectroscopy (NMR) (Moir and Bowman, 1993; Turner et al., 2003), were carried out on composite soil samples collected from the 0-5 cm layer in 1999, 2004 and 2009 for 4 replicates under *Pinus radiata*. This involved shaking 5 g of soil with 100 mL of a solution containing 0.25 M sodium hydroxide and 0.05 M EDTA for 16 h at 20**°**C, and then carrying out NMR analysis of reconstituted freeze dried extracts. NMR analyses were conducted in the Soils Laboratory of the Smithsonian Tropical Research Institute, Panama.

4.2.1.2 Statistical Analysis

All statistical analysis were performed using the R program version 2.15.0 (2012, The R Foundation for Statistical Computing). One and two way analyses of variance (ANOVA) were carried out on the data to test the significance of effects tree species (*Pinus radiata*, *Cupressus macrocarpa* and *Eucalyptus nitens*), soil depths (0-5, 5-10 and 10-20 cm) and time (1999, 2004 and 2009) on soil properties. One way ANOVA was also carried out to test the temporal effects on the enzyme lability and NMR properties on the soil. Where F ratios were significant ($P< 0.05$), treatment means were compared by the Least Significant Difference (*lsd*) test in a general ANOVA.

4.2.2 Results

4.2.2.1 Soil P Fractionation

Table 4.2.1 shows data for the concentrations of labile P, $NaHCO_3$ P_o, NaOH-I P_i, NaOH-I P_o, HCl P_i, NaOH-II P_i, NaOH-II P_o, total extractable inorganic P (ΣPi) and total extractable organic P (ΣPo) fractions under *P. radiata*, *C. macrocarpa* and *E. nitens* as determined for each depth (0-5, 5-10 and 10-20 cm), in the years 1999, 2004 and 2009. Degrees of freedom and associated P values can be seen in Table 4.2.2.

On average, the labile P, NaOH-I P_i, HCl P_i and NaOH-II P_i fractions represented 14, 57, 16 and 13% of total extractable inorganic P, respectively, while the $NaHCO_3$ P_o, NaOH-I P_o and NaOH-II P_o fractions represented 17, 57 and 26% of total extractable Po, respectively.

While the concentrations of labile P, $NaHCO_3$ P_o, HCl P_i consistently decreased with depth under all species in all years, the concentrations of NaOH-I P_o, NaOH-II P_i and NaOH-II P_o did not show consistent differences between soil depths. On the other hand, NaOH-I P_i increased with depth under *P. radiata* and *C. macrocarpa* but not under *E. nitens* in 1999 (Table 4.2.1).

Table 4.2.1 Mean concentrations (mg P/ kg) of labile P, $NaHCO_3$ P_o, NaOH-I P_i, NaOH-I P_o, HCl P_i, NaOH-II P_i, NaOH-II P_o and total extractable P_i and P_o fractions under *P. radiata*, *C. macrocarpa* and *E. nitens* at 3 depths (0-5, 5-10 and 10-20 cm) in 1999, 2004 and 2009. Data in columns are means (n=4); data in parenthesis are standard errors of means.

	Depth	Year	Labile P	$NaHCO_3$ P_o	NaOH-I P_i	NaOH-I P_o	HCl P_i	NaOH-II P_i	NaOH-II P_o	ΣP_i	ΣP_o	ΣP
			(mg P/ kg)									
P. radiata	0-5											
		1999	41.0	90.7	114.2	252.1	43.5	25.1	88.7	223.9	431.5	655.4
			(2.3)	*(5.4)*	*(21.3)*	*(15.3)*	*(1.5)*	*(2.4)*	*(8.6)*	*(18.1)*	*(20.5)*	*(38.6)*
		2004	40.2	40.4	125.8	177.3	38.5	22.6	79.8	227.0	297.5	524.5
			(8.0)	*(8.5)*	*(14.6)*	*(10.1)*	*(0.4)*	*(1.2)*	*(4.4)*	*(16.4)*	*(15.7)*	*(32.1)*
		2009	36.0	85.6	98.3	194.9	31.8	40.0	114.7	206.0	395.2	601.2
			(3.0)	*(4.1)*	*(10.2)*	*(7.8)*	*(2.4)*	*(4.8)*	*(14.9)*	*(12.7)*	*(25.3)*	*(38.0)*
	5-10											
		1999	28.8	78.0	152.1	227.8	39.9	25.6	91.7	246.4	397.6	644.0
			(1.6)	*(9.7)*	*(18.0)*	*(47.8)*	*(2.6)*	*(1.5)*	*(5.4)*	*(14.8)*	*(59.0)*	*(73.8)*
		2004	34.8	30.8	151.3	185.7	39.5	22.5	80.5	248.1	297.1	545.2
			(6.0)	*(3.6)*	*(13.8)*	*(17.8)*	*(0.9)*	*(1.1)*	*(3.9)*	*(18.1)*	*(22.1)*	*(40.2)*
		2009	28.2	71.8	114.7	204.1	35.3	44.3	96.9	222.6	372.8	595.4
			(2.4)	*(4.3)*	*(18.0)*	*(18.0)*	*(2.2)*	*(7.9)*	*(6.9)*	*(24.7)*	*(15.8)*	*(40.5)*
	10-20											
		1999	20.7	58.7	140.6	231.7	39.9	24.4	91.5	225.6	381.8	607.4
			(0.8)	*(6.8)*	*(7.4)*	*(35.4)*	*(4.8)*	*(2.1)*	*(8.0)*	*(14.6)*	*(48.2)*	*(62.8)*
		2004	24.6	21.3	147.5	185.3	38.7	22.4	83.6	233.2	290.2	523.4
			(7.0)	*(7.8)*	*(18.2)*	*(21.8)*	*(3.8)*	*(1.2)*	*(4.4)*	*(25.3)*	*(29.0)*	*(54.3)*
		2009	19.4	48.3	145.3	185.1	28.2	41.8	93.5	234.7	326.9	561.6
			(1.3)	*(6.8)*	*(7.5)*	*(11.8)*	*(3.3)*	*(7.2)*	*(10.7)*	*(17.1)*	*(22.6)*	*(39.7)*
C. macrocarpa												
	0-5											
		1999	45.2	94.3	120.4	220.0	43.2	26.4	93.4	235.3	407.7	643.0
			(4.3)	*(11.2)*	*(21.1)*	*(18.8)*	*(4.7)*	*(1.7)*	*(5.9)*	*(30.7)*	*(22.2)*	*(52.9)*
		2004	44.2	41.3	122.2	186.0	36.6	22.8	80.5	225.8	307.8	533.6
			(5.2)	*(3.8)*	*(22.7)*	*(22.9)*	*(6.1)*	*(2.6)*	*(9.1)*	*(35.1)*	*(24.9)*	*(60.0)*
		2009	45.6	79.8	120.3	168.6	32.6	30.9	99.9	229.4	348.3	577.7
			(5.1)	*(12.8)*	*(19.6)*	*(12.3)*	*(5.4)*	*(3.1)*	*(6.4)*	*(30.9)*	*(27.2)*	*(58.1)*
	5-10											
		1999	32.3	88.0	121.1	204.7	38.3	25.9	92.9	217.6	385.7	603.3
			(2.3)	*(9.7)*	*(13.1)*	*(13.3)*	*(6.0)*	*(2.0)*	*(7.0)*	*(21.8)*	*(14.4)*	*(36.2)*
		2004	31.9	37.6	117.1	215.8	35.7	22.5	80.5	207.2	333.9	541.1
			(4.6)	*(4.7)*	*(18.0)*	*(26.6)*	*(6.9)*	*(2.0)*	*(7.0)*	*(29.2)*	*(34.5)*	*(63.7)*
		2009	32.1	74.9	128.1	193.3	32.0	40.2	92.9	232.4	361.1	593.5
			(2.3)	*(11.2)*	*(13.2)*	*(27.3)*	*(5.6)*	*(7.1)*	*(9.7)*	*(27.2)*	*(36.4)*	*(63.6)*
	10-20											
		1999	23.5	55.6	140.2	243.7	38.0	25.4	95.0	227.1	394.2	621.3
			(0.9)	*(10.0)*	*(17.7)*	*(15.3)*	*(8.5)*	*(1.9)*	*(7.0)*	*(29.0)*	*(28.1)*	*(57.1)*
		2004	23.2	24.5	139.8	202.4	33.1	21.5	80.3	217.6	307.1	524.7
			(5.9)	*(3.9)*	*(12.8)*	*(25.7)*	*(7.8)*	*(2.9)*	*(11.0)*	*(26.6)*	*(36.2)*	*(62.8)*
		2009	21.2	55.1	133.8	182.6	28.4	40.4	107.5	223.9	345.2	569.1
			(0.4)	*(6.6)*	*(16.4)*	*(22.5)*	*(4.9)*	*(6.5)*	*(7.9)*	*(26.2)*	*(27.5)*	*(53.7)*
E. nitens												
	0-5											
		1999	40.9	98.5	135.2	217.3	41.3	23.2	82.1	240.7	397.9	638.6
			(2.0)	*(13.6)*	*(27.1)*	*(13.0)*	*(2.7)*	*(2.2)*	*(7.8)*	*(29.2)*	*(18.2)*	*(47.4)*
		2004	45.8	43.4	110.4	178.2	38.3	23.3	82.4	217.8	304.1	521.9
			(5.8)	*(5.3)*	*(13.2)*	*(25.4)*	*(4.4)*	*(1.7)*	*(6.1)*	*(23.0)*	*(29.5)*	*(52.5)*
		2009	38.1	75.5	96.7	177.6	29.3	33.0	98.0	197.2	351.1	548.3
			(3.6)	*(5.8)*	*(10.6)*	*(6.2)*	*(4.2)*	*(4.7)*	*(5.6)*	*(21.3)*	*(10.8)*	*(32.1)*
	5-10											
		1999	30.3	89.1	111.1	236.0	35.4	24.7	88.4	201.5	413.5	615.0
			(0.8)	*(13.8)*	*(13.1)*	*(22.4)*	*(6.7)*	*(2.3)*	*(8.3)*	*(21.3)*	*(36.2)*	*(57.5)*
		2004	37.7	35.1	133.9	181.7	38.0	23.7	84.8	233.2	301.7	534.9
			(8.2)	*(5.8)*	*(11.6)*	*(26.7)*	*(4.2)*	*(2.6)*	*(9.4)*	*(25.2)*	*(37.3)*	*(62.5)*
		2009	30.0	66.1	99.9	179.5	29.9	35.2	103.1	195.0	348.6	543.6
			(2.7)	*(4.8)*	*(9.9)*	*(16.0)*	*(5.1)*	*(6.0)*	*(6.4)*	*(18.1)*	*(21.1)*	*(39.2)*
	10-20											
		1999	21.2	72.5	129.2	222.7	36.1	24.3	91.0	210.8	386.1	596.9
			(0.2)	*(10.7)*	*(18.5)*	*(16.8)*	*(6.4)*	*(2.2)*	*(8.1)*	*(27.5)*	*(24.1)*	*(51.6)*
		2004	29.1	21.9	165.4	150.9	39.9	23.4	87.3	257.8	260.1	517.9
			(7.2)	*(9.5)*	*(22.9)*	*(9.3)*	*(6.9)*	*(2.3)*	*(8.5)*	*(34.4)*	*(21.4)*	*(55.8)*
		2009	21.7	45.3	109.7	185.0	27.4	38.1	90.7	196.8	321.1	517.9
			(1.9)	*(3.8)*	*(15.1)*	*(8.5)*	*(5.1)*	*(11.8)*	*(9.5)*	*(22.4)*	*(13.2)*	*(35.6)*

*Labile P is the sum of inorganic and organic P in NaCl; **ΣP_i is the sum of inorganic P in NaCl, $NaHCO_3$, NaOH-I, HCl, and NaOH-II; ***ΣP_o is the sum of organic P in NaCl, $NaHCO_3$, NaOH-I and NaOH-II, **** ΣP is the sum of $\Sigma P_i + \Sigma P_o$.

Table 4.2.2 shows the ANOVA F values and their levels of significance for all of the soil P fractions for the three different species and years of sampling, together with the corresponding interaction. There were no significant differences observed between the three tree species for any of the soil P fractions and species-year interactions, although significant differences with time were found for $NaHCO_3$ P_o, NaOH-I P_o, NaOH-II P_i, NaOH-II P_o and total extractable P_o.

Table 4.2.2 F values with levels of significance from analyses of variance for all the soil P fractions for species and year and the interaction between species and year (Sp x Y) for 0-5, 5-10 and 10-20 cm. Numbers in **bold** show significant differences (p <0.05).

		Source of variation					
Depth		Species (Sp)		Year (Y)		Sp x Y	
	Degrees of freedom→	F	*p*	F	*p*	F	*p*
0-5	Labile P	1.209	0.314	0.434	0.652	0.296	0.878
	$NaHCO_3$ P_o	0.955	0.505	30.109	**< 0.001**	0.292	0.881
	NaOH-I P_i	0.166	0.848	0.791	0.463	0.427	0.788
	NaOH-I P_o	1.120	0.341	9.644	**< 0.001**	0.581	0.679
	HCl P_i	0.135	0.875	6.263	**< 0.006**	0.106	0.979
	NaOH-II P_i	0.770	0.473	13.420	**< 0.001**	1.054	0.398
	NaOH-II P_o	0.533	0.593	6.385	**< 0.005**	0.615	0.656
	ΣP_i	0.211	0.811	0.612	0.550	0.198	0.937
	ΣP_o	0.883	0.425	19.431	**< 0.001**	0.545	0.704
5-10	Labile P	0.193	0.826	1.139	0.335	0.331	0.854
	$NaHCO_3$ P_o	0.486	0.620	29.295	**< 0.001**	0.266	0.897
	NaOH-I P_i	2.225	0.128	1.466	0.249	1.124	0.366
	NaOH-I P_o	0.288	0.943	1.305	0.059	0.539	0.708
	HCl P_i	0.498	0.613	1.205	0.095	0.115	0.983
	NaOH-II P_i	0.357	0.703	13.292	**< 0.001**	0.403	0.804
	NaOH-II P_o	0.163	0.850	3.474	**< 0.001**	0.322	0.861
	ΣP_i	1.281	0.294	0.239	0.789	0.633	0.643
	ΣP_o	0.114	0.986	5.854	**< 0.001**	0.322	0.861
10-20	Labile P	0.284	0.755	1.246	0.304	0.277	0.890
	$NaHCO_3$ P_o	0.186	0.832	20.840	**< 0.001**	0.827	0.520
	NaOH-I P_i	0.288	0.752	1.395	0.265	0.930	0.461
	NaOH-I P_o	1.013	0.377	6.300	**< 0.001**	0.465	0.760
	HCl P_i	0.124	0.884	2.603	0.093	0.179	0.947
	NaOH-II P_i	0.023	0.977	9.576	**< 0.001**	0.071	0.990
	NaOH-II P_o	0.294	0.748	1.933	0.064	0.523	0.719
	ΣP_i	0.124	0.884	0.429	0.655	0.625	0.649
	ΣP_o	0.764	0.475	10.743	**< 0.001**	0.158	0.958

Accordingly, data for the different fractions and total extractable inorganic P and organic P were averaged across all tree species to examine the effect of sampling year on P concentrations. Results for samples taken in 1999, 2004 and 2009 from 0-5 cm, 5-10 cm and 10-20 cm are shown in Figures 4.2.5, 4.2.6 and 4.2.7, respectively.

In general, the changes observed in each fraction and total extractable pools over the different sampling dates were similar for all depths. Labile P and NaOH-I P_i and total extractable inorganic P did not show significant changes between years at any depth, while HCl P_i decreased significantly between 2004 and 2009. On the other hand, NaOH-II P_i increased significantly, again at all depths, between 2004 and 2009 (Tables 4.2.1 and 4.2.2).

For all soil depths, $NaHCO_3$ P_o decreased significantly between 1999 and 2004, but then increased significantly between 2004 and 2009, although the overall trend between 1999 and 2009 is statistically significant. Concentrations of NaOH-I P_o also decreased significantly between 1999 and 2004, but were similar in 2009 compared with 2004. Conversely, NaOH-II P_o did not change between 1999 and 2004 but increased significantly between 2004 and 2009. Total extractable P_o decreased significantly between 1999 and 2004, but then increased significantly between 2004 and 2009, although the overall decrease that occurred between 1999 and 2009 remained significant (Tables 4.2.1 and 4.2.2).

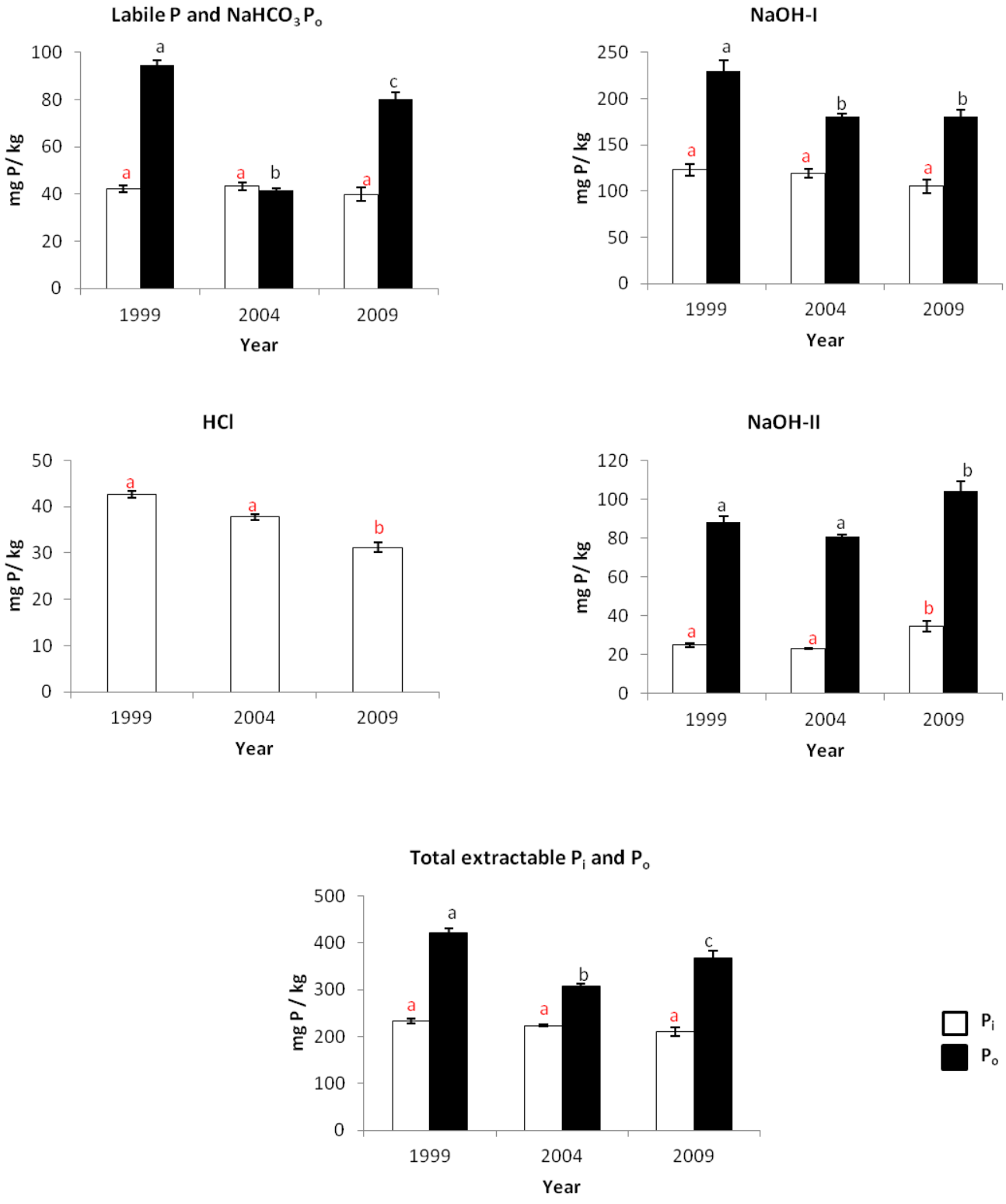

Figure 4.2.5 Mean concentrations (mg P/kg) in different P fractions determined in 1999, 2004 and 2009 for 0-5 cm averaged over the three tree species (*P. radiata*, *C. macrocarpa* and *E. nitens)*. The error bars represent the standard errors of means. Different letters indicate that means were significantly different between sampling years ($p < 0.05$).

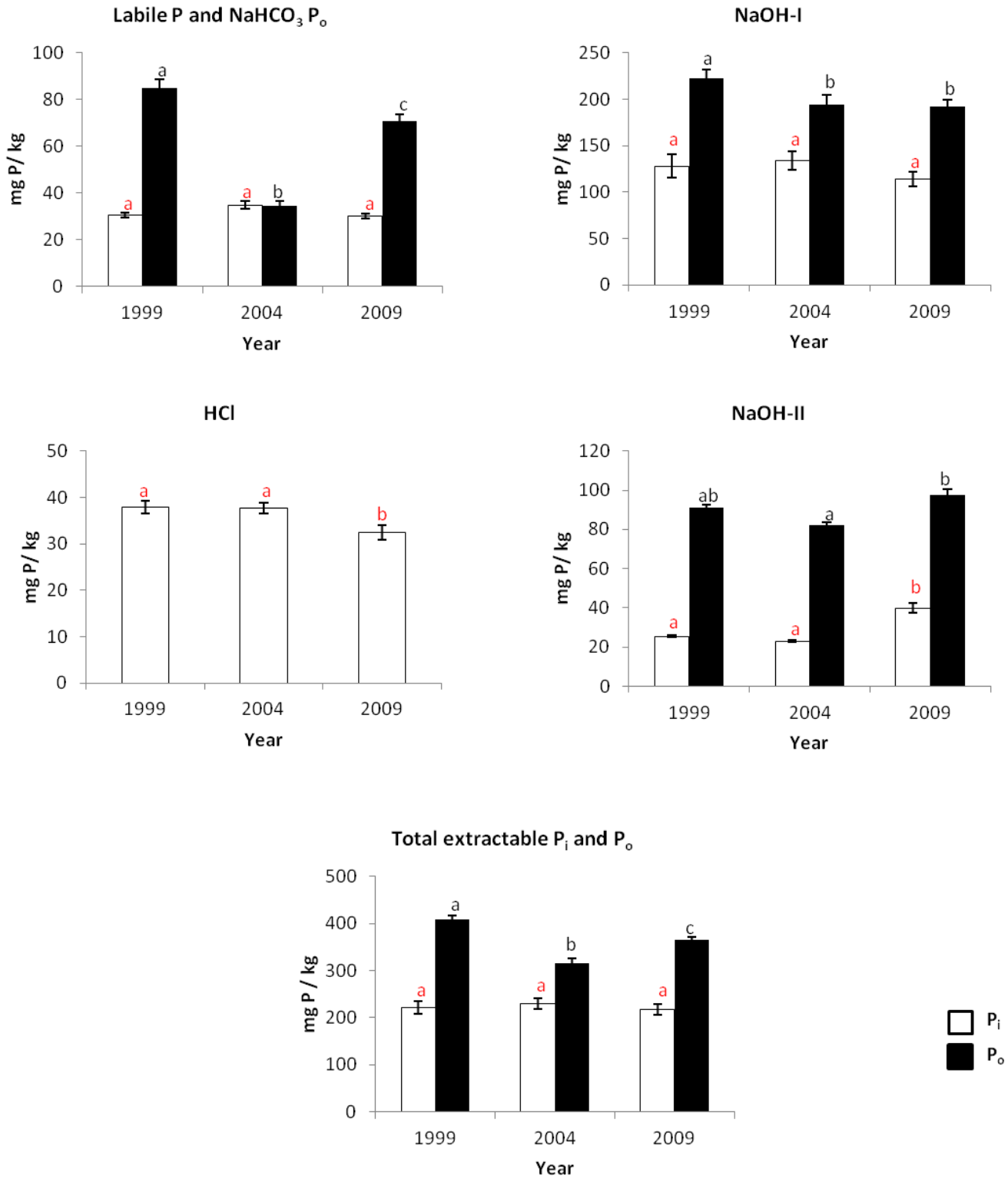

Figure 4.2.6 Mean concentrations of P (mg P/kg) in different P fractions determined in 1999, 2004 and 2009 for 5-10 cm averaged over the three tree species (*P. radiata*, *C. macrocarpa* and *E. nitens)*. The error bars represent the standard errors of means. Different letters indicate that means were significantly different between sampling years ($p < 0.05$).

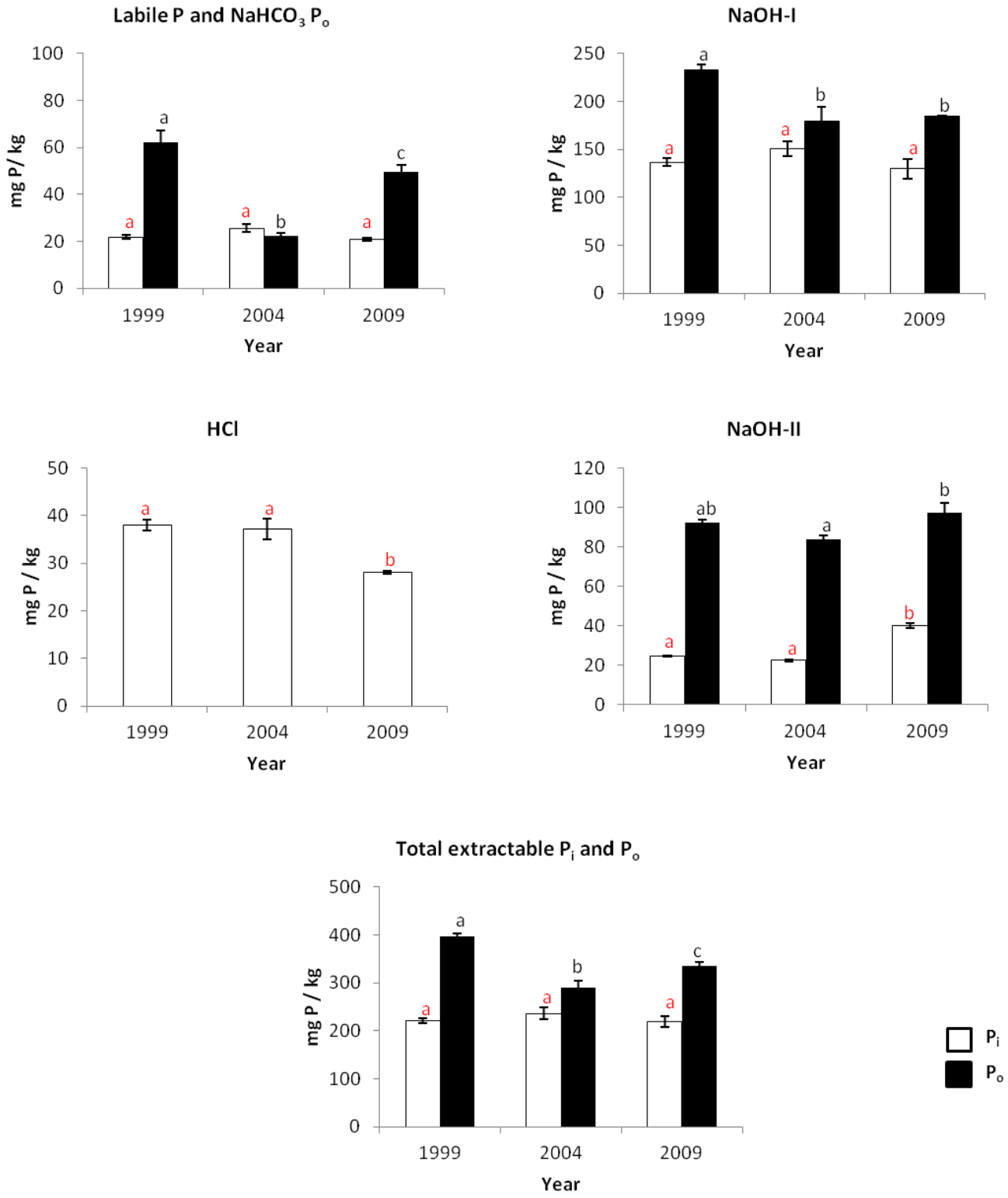

Figure 4.2.7 Mean concentrations of P (mg P/kg) in different P fractions determined in 1999, 2004 and 2009 for 10-20 cm averaged over the three tree species (*P. radiata*, *C. macrocarpa* and *E. nitens)*. The error bars represent the standard errors of means. Different letters indicate that means were significantly different between sampling years ($p < 0.05$).

4.2.2.2 Enzyme-labile soil organic P

Data for enzyme labile $NaHCO_3$-extractable organic P determined for the 0-5 cm soil under *Pinus radiata* in 1999, 2004 and 2009 are shown in Figure 4.2.8. Results showed that inorganic P release was consistently higher for alkaline phosphatase (50-70 mg P/kg) compared with phytase (30-35 mg P/kg), which reflected the respective quantities of organic P hydrolysed by each enzyme. Alkaline phosphatase hydrolysable P decreased significantly between 2004 and 2009 from 50 to70 mg P/kg, while phytase hydrolysable P increased between 1999 and 2004 and the decreased between 2004 and 2009.

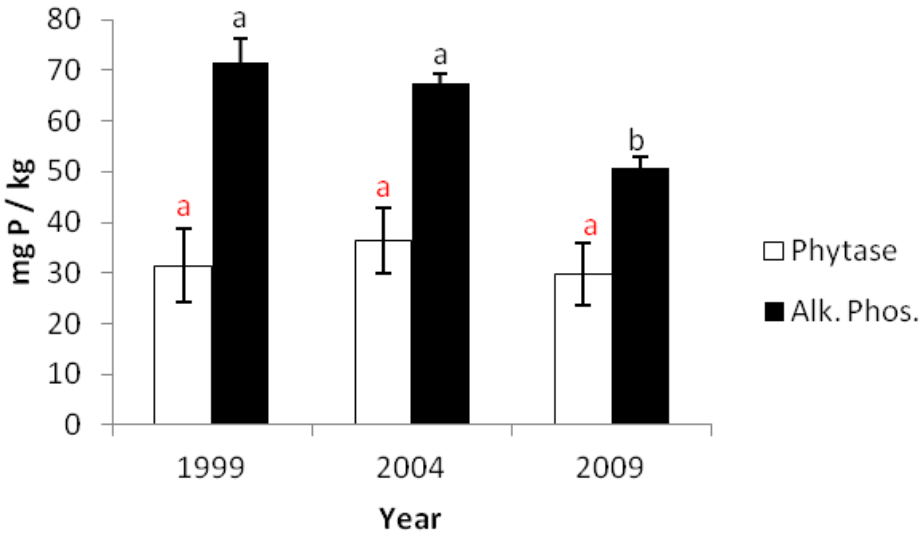

Figure 4.2.8 Mean concentrations of inorganic P (mg P/kg) released by phytase and alkaline phosphatase in 1999, 2004 and 2009 under *P. radiata* for 0-5 cm soil. The error bars represent the standard errors of means. Different letters indicate that means were significantly different between sampling years ($p < 0.05$).

4.2.2.3 ^{31}P NMR

Table 4.2.3 shows the concentration of organic P compounds determined by ^{31}P NMR in extracts of 0-5 cm soils under radiata pine sampled in 1999, 2004 and 2009. As expected phosphate monoesters accounted for 90-95% of the organic P detected, and these decreased with time from 408 mg P/kg in 1999 to 343 mg P/kg in 2009. This was mainly due to decreases in the “other monoester” fraction, while the only other change noted was a 10-fold increase in phosphonate-P between 2004 and 2009.

Table 4.2.3 Concentrations (mg P/kg) of organic P compounds determined by ^{31}P NMR in NaOH-EDTA extracts of 0-5 cm soils under radiata pine sampled in 1999, 2004 and 2009. Values in parentheses are the proportion (%) of the total phosphorus extracted by NaOH-EDTA.

Year	Organic P (mg P/kg)					
	Phosphate monoesters			Phosphate diesters		Phosphonates
	myo-IP_6	*scyllo*-IP_6	Other	DNA	Phospholipids	
1999	56 (11)	20 (4)	332 (49)	6 (1)	5(1)	5 (1)
2004	63 (13)	34 (7)	281 (37)	2 (<1)	5 (1)	2 (<1)
2009	57 (12)	29 (6)	257 (36)	8 (2)	5 (1)	20 (4)

4.2.3 Discussion

It was hypothesised that the quantities and nature of soil P would change during the 10 years following afforestation and that any changes in soil P would be affected by tree species. However, except for a lower concentration of $NaHCO_3$ P_o found for 0-5 cm soil under *P. radiata*, the three tree species had a very similar overall impact on the amounts and forms of P determined in the soil to a depth of 20 cm over the first 10 years following establishment.

This was particularly surprising given the different growth rates of the three tree species (Table 4.2.4), which were clearly demonstrated in their different impact on the grassland understorey. After 5 years (2004), it was clear that *P. radiata* has markedly reduced understorey grass growth (Figure 4.2.9) which was probably mainly attributable to a combination of shading, as a consequence of canopy development, and also competition for available soil water. On the other hand, while *E. nitens* caused less shading, the grassland understory had almost disappeared by 2004, which may have been due to competition for soil moisture by the rapidly growing trees. Over the same period, the grassland understory persisted to a greater extent under *C. macrocarpa*, which probably reflected slower growth rates compared with *P. radiata* and *E. nitens* (Figure 4.2.9).

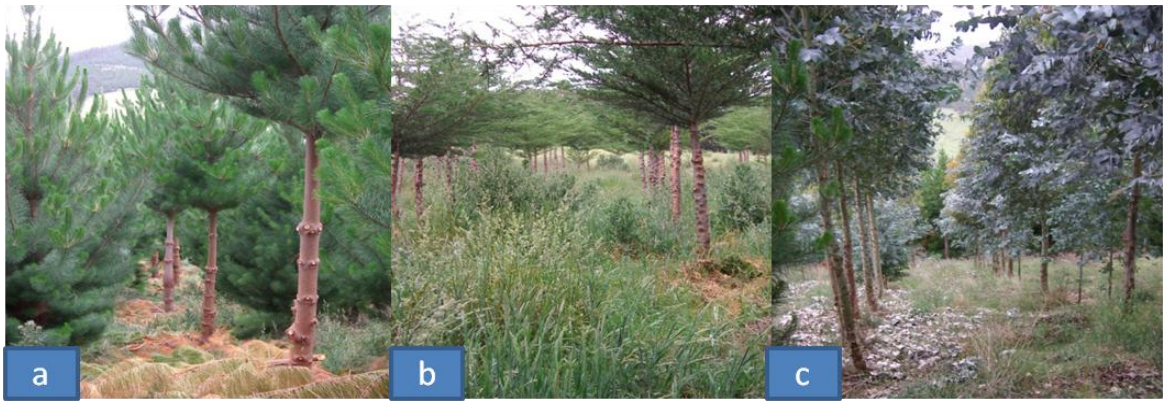

Figure 4.2.9 Status of the understory under a) *Pinus radiata*, b)*Cupressus macrocarpa* and c) *Eucalyptus nitens* in 2004.

By 2009 (10 years), complete canopy closure was evident in the *P. radiata* plots and the grassland understory had been effectively buried under a combination of needle litter and course wood debris from thinning and pruning (Figure 4.2.10). There was no grassland understory evident under *E. nitens*, while it has diminished substantially under *C. macrocarpa* (although still present).

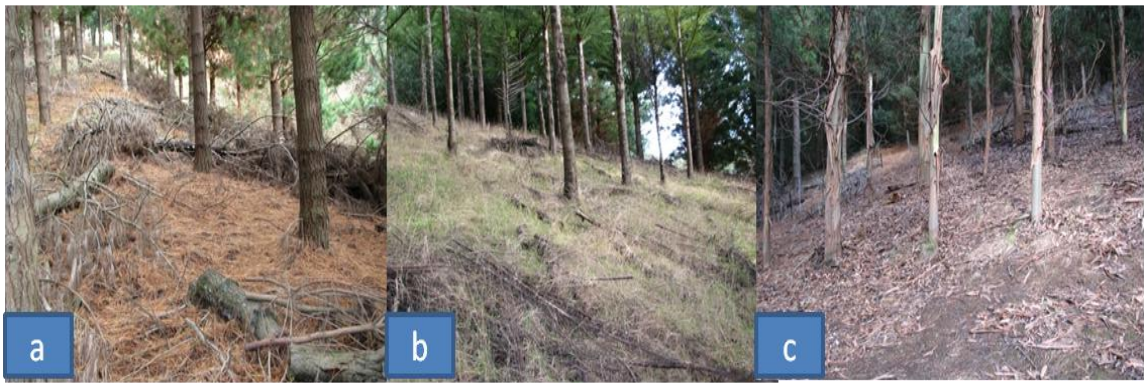

Figure 4.2.10 Status of the understory under a) *Pinus radiata*, b)*Cupressus macrocarpa* and c) *Eucalyptus nitens* in 2009.

The fact that $NaHCO_3$ P_o, NaOH-I P_o and total extractable P_o decreased significantly between 1999 and 2004 indicated that forest establishment had resulted in net mineralization of soil organic P. This is consistent with previous paired site comparison studies which showed that significant mineralization of soil organic P occurred 10-20 years after the change in land-use (Chen et al., 2000; Condron et al., 1996; Davis and Lang, 1991; Hawke and O'Connor, 1993) , although the fact that soil organic P depletion occurred during the first 5 years after tree planting is a significant new finding to emerge from this study.

It is also possible that the significant decrease observed between 1999 and 2004 could be associated with bulk density changes promoted by the growth of tree roots, but unfortunately there is not data available to be compared between those years. Another explanation is that the organic forms of P were relocated in a non extractable pool that was not observed through the fractionation method used here, however, the percentage of the organic fractions with respect to the total extractable P_o showed a similar trend than that for concentrations (Table 4.2.4). Differences associated with soil sampling between years that altered the amount of total P would also have explained this behaviour.

Table 4.2.4 Mean percentage (%) of labile P, $NaHCO_3$ P_o, NaOH-I P_i, NaOH-I P_o, HCl P_i, NaOH-II P_i, and NaOH-II P_o and mean concentration (mg P/kg) of total extractable P_i and P_o fractions under *P. radiata*, *C. macrocarpa* and *E. nitens* at 3 depths (0-5, 5-10 and 10-20 cm) in 1999, 2004 and 2009.

	Depth	Year	Labile P	NaHCO3-Po	NaOH1-Pi	NaOH1-Po	HCl-Pi	NaOH2-Pi	NaOH2-Po	Σ Pi	Σ Po
						(%)				(mg P/kg	
P. radiata	0-5	1999	18	21	51	58	19	11	21	223.9	431.5
		2004	18	14	55	60	17	10	27	227.0	297.5
		2009	17	22	48	49	15	19	29	206.0	395.2
	5-10	1999	12	20	62	57	16	10	23	246.4	397.6
		2004	14	10	61	63	16	9	27	248.1	297.1
		2009	13	19	52	55	16	20	26	222.6	372.8
	10-20	1999	9	15	62	61	18	11	24	225.6	381.8
		2004	11	7	63	64	17	10	29	233.2	290.2
		2009	8	15	62	57	12	18	29	234.7	326.9
C. macrocarpa											
	0-5	1999	19	23	51	54	18	11	23	235.3	407.7
		2004	20	13	54	60	16	10	26	225.8	307.8
		2009	20	23	52	48	14	13	29	229.4	348.3
	5-10	1999	15	23	56	53	18	12	24	217.6	385.7
		2004	15	11	57	65	17	11	24	207.2	333.9
		2009	14	21	55	54	14	17	26	232.4	361.1
	10-20	1999	10	14	62	62	17	11	24	227.1	394.2
		2004	11	8	64	66	15	10	26	217.6	307.1
		2009	9	16	60	53	13	18	31	223.9	345.2
E. nitens											
	0-5	1999	17	25	56	55	17	10	21	240.7	397.9
		2004	21	14	51	59	18	11	27	217.8	304.1
		2009	19	21	49	51	15	17	28	197.2	351.1
	5-10	1999	15	22	55	57	18	12	21	201.5	413.5
		2004	16	12	57	60	16	10	28	233.2	301.7
		2009	15	19	51	51	15	18	30	195.0	348.6
	10-20	1999	10	19	61	58	17	12	24	210.8	386.1
		2004	11	8	64	58	15	9	34	257.8	260.1
		2009	11	14	56	58	14	19	28	196.8	321.1

*Labile P is the sum of inorganic and organic P in NaCl; **ΣP_i is the sum of inorganic P in NaCl, $NaHCO_3$, NaOH-I, HCl, and NaOH-II; ***ΣP_o is the sum of organic P in NaCl, $NaHCO_3$, NaOH-I and NaOH-II, **** ΣP is the sum of $\Sigma P_i + \Sigma P_o$.

Phosphorus uptake by newly established trees may at least partly explain the enhanced mineralization of soil organic P, although it was surprising that this was similar under all three tree species despite differences in mycorrhizal associations, root physiology and growth rates. In particular, it was expected that differences in mycorrhizae between *P. radiata* (ectomycorrhizae [EM]), *C. macrocarpa* (vesicular-arbuscular mycorrhizae [VAM]) and *E. nitens* (EM/VAM) would result in different impacts on soil P. Previous studies have showed that short-term mineralization of rhizosphere soil organic P was significantly greater for radiata pine compared with perennial ryegrass (*Lolium perenne*) which has VAM (Chen et al., 2002). The fact that soil organic P decreases were similar under all three tree species suggests that EM and VAM were equally effective at mobilising organic P by mineralisation, which is contrary to current understanding of the role of different mycorrhizal types in P acquisition (Chen et al., 2002; Lambers et al., 2008; Read and Perez-Moreno, 2003; Smith et al., 2008; Smith and Read, 2008).

Tree biomass measurements taken in 2009 showed that standing stem volumes (m^3/ha) were greater for *P. radiata* (211) and *E. nitens* (165) compared with *C. macrocarpa* (109) (Table 4.2.5). There was no corresponding data available for P content of the tree biomass, but the fact that changes in soil P were similar for all three tree species may indicate comparable quantities of biomass P uptake.

Table 4.2.5 Comparative growth of the three tree species determined 10 years after planting (2009) (standard errors are shown in parenthesis) (Davis et al., 2010).

Species	Diameter (cm)	Height (m)	Basal area (m^2/ha)	Stem volume (m^3/ha)
Pinus radiata	28.5	16.7	33.9	211
	(0.41)	(0.43)	(0.73)	(4.1)
Eucalyptus nitens	20.7	16.7	25.1	165
	(2.08)	(1.04)	(2.66)	(11.9)
Cupressus macrocarpa	17.5	10.8	24.2	109
	(0.99)	(0.29)	(1.29)	(5.8)

It is also possible that changes in P cycling associated with the cessation of grazing following tree planting could have contributed to the immediate significant decline in soil organic P. This in turn may be attributed to changes in the quantities of P being returned to soil and its consequent impacts on the balance between the respective rates of organic P inputs and turnover, especially since the $NaHCO_3$ and NaOH-I P fractions are generally considered to represent the more labile pools of soil organic P (Condron et al., 2005). This suggests that the quantity and turnover of organic P in soil may have been reduced following the cessation of grazing. Grazing animals play a very important role in the process of P cycling in pasture ecosystems since 95% of the P taken up by plants is returned to the soil in the form of feces and root residues, while only 25% of ingested P is commonly removed by transfer of animal excreta within the system (Haynes and Williams, 1993; Kemp et al., 2000). Simpson et al. (2012) investigated the relative solubility of soil P under contrasting mowing regimes (no mowing, clippings removed, clippings left) which had been maintained in a field trial for 15 years. In approximate terms, the regime where clippings were left was equivalent to grazing while no mowing would be equivalent the situation at Orton Bradley Park following the cessation of grazing when trees were planted. They showed that biological and biochemical processes associated with enhanced mineralization of organic P were significantly greater in soils where were clipping left compared to no mowing, which reflected increased organic matter inputs under the clippings return regime. In addition, studies of long-term changes in soil P under grazed pasture in New Zealand and elsewhere have clearly and consistently demonstrated that concentrations of soil organic P increased significantly with time. This was attributed to elevated rates of organic matter and P inputs and turnover compared with soils under unimproved and native vegetation (Condron and Goh, 1989a; Condron and Goh, 1989b; Condron et al., 2005; McDowell and Condron, 2012).

While a significant increase in soil organic P was observed between 2004 and 2009, it is important to note that the concentrations of total extracted P_o in 2009 were still significantly lower than 1999. Nonetheless, increases in $NaHCO_3$ P_o and concomitant significant increases in NaOH II-P_o over this period may also reflect changes in

organic P inputs and dynamics compared with the 1999-2004 period. While the observed increase in $NaHCO_3$ P_o between 2004 and 2009 indicates an increase in labile Po, this may in fact be related to an overall reduction in soil microbial biomass and activity as the forest develops. Previous paired-site studies clearly showed that both the size and activity of the soil microbial biomass, together with phosphatase enzyme activities, were consistently and significantly lower under forest compared with adjacent grassland (Chen et al., 2003; Chen et al., 2008). He et al. (1997) explained that fluctuations in the size and turnover of soil microbial biomass is very important to control the turnover of C, N and P, which in turn regulate plant availability of P. Chen et al. (2003) found that concentrations of microbial biomass P were higher in grasslands compared to adjacent forest and concluded that it was mainly affected by returns of grass root litter in the first and by litterfall accumulation in the second. Increases in non-labile NaOH-II P_o that occurred between 2004 and 2009 may indicate a shift towards more recalcitrant organic matter in soil reflecting increased inputs of organic matter and P from tree residues and roots. Huang et al. (2011) analysed soils taken from the Orton Bradley Park trial over the same period (1999-2009) to quantify changes in soil C mass in light and heavy fractions. Although they found that C mass did not show significant differences in the soil heavy fraction, C mass in the light fraction displayed a similar pattern to that found in this study for $NaHCO_3$ P_o (labile and most abundant) and NaOH-II P_o (most recalcitrant) by decreasing its concentration between 1999 and 2004 and increasing by 2009. They attributed the decrease at year 5 to reduced C inputs from grassland litter, while the increased of C mass between year 5 and 10 was due to C inputs from tree residues. Condron and Newman (1998) used ^{13}C-NMR spectroscopy to investigate the chemical nature of the soil organic C under grassland and adjacent recently established coniferous forests (10-17 years). They found that under grassland the soil organic C was closely related to plant fragments and partially degraded residues derived from grass roots, while soil organic C was more recalcitrant under forest These changes in soil organic C are consistent with the increases in the recalcitrant NaOH-II P_o fraction that was observed between 2004 and 2009. As the turnover of C in a system increases through plant addition, the biological processes of mineralization determine the availability of P (Bünemann and

Condron, 2007; Richardson et al., 2004). Furthermore, labile organic P could be mineralised by greater microbial activity to meet increasing plant demand in the early stages of tree establishment.

Specific results determined for changes in enzyme hydrolysable P and organic P under *P. radiata* over 10 years provided some important additional perspect on the nature of changes that occurred following tree planting. The fact that alkaline phosphatase hydrolysable P was similar in 2004 compared to 1999 was surprising given that $NaHCO_3$ extractable P_o decreased significantly over the same period. On the other hand, alkaline phosphatase hydrolysable P decreased significantly between 2004 and 2009, while $NaHCO_3$ P_o actually increased significantly over this time. These findings indicate that while the solubility of organic P in $NaHCO_3$ increased between 2004 and 2009, its susceptibility to enzyme mineralisation (i.e. its lability) actually decreased. The latter may indicate a shift towards more recalcitrant soil organic matter between 2004 and 2009 as discussed above. However, differences in soil organic P solubility and enzyme lability also highlights limitations in assigning relative lability/stability based on ease of extraction from soil (Condron and Newman, 2011). The NMR data showed that monoester forms of organic P decreased as a consequence of afforestation of grassland , which is consistent with studies which compared changes in soil organic P under radiata pine-and perennial ryegrass (Turner et al., 2005). However, the decreases in organic P determined in the present study were confined to “other monoesters”, while the other studies noted significant depletion of *scyllo* and *myo* inositol hexakisphosphates. This may be at least partly related to the fact that the comparative studies cited above were carried out on soils from a 10 month glasshouse pot trial, as opposed to changes determined over 10 years under field conditions in the present study. The occurrence of phosphonates P in soil is linked to acidity (Condron et al., 2005), and the increase observed between 2004 and 2009 may be at least partly be attributed to a decline in soil pH on the trial from 4.9 in 1999 to 4.4-4.5 in 2009. This pH decline under trees may also account for the significant decrease in HCl P_i that occurred between 1999 and 2009 (Figure 4.2.2).

4.3 Orton Bradley Park Seasonal Study

A seasonal investigation was conducted over a 12 month period in 2011-12 (12-13 years after establishment) to determine the short term effects of the tree species on the chemical nature, dynamics and bioavailability of soil P. It was hypothesised that tree species would have a significant impact on temporal and seasonal differences in the nature and availability of soil P as a consequence of different growth and P acquisition strategies.

4.3.1 Materials and Methods

4.3.1.1 Research site and soil sampling

Samples of mineral soil (0-5 cm) were taken from all plots at the Orton Bradley Park trial, using an Ellenkamp corer (6 cm diameter) from five sites within each replicate plot, and bulked, over five seasons: April 2011 (autumn I), July 2011 (winter), October 2011 (spring), January 2012 (summer) and April 2012 (autumn II). Soil samples were sieved < 4 mm and stored at 4 °C prior to analysis, and sub-samples were air-dried at 30 °C, finely ground (< 150 µm) and stored in plastic containers.

4.3.1.2 Soil analysis

Concentrations of labile monoesters released by alkaline phosphatase and phytase were determined for <4 mm field moist soil within 72 hours of collection using the methods described above (4.2.1.1). Results are expressed on an air-dried soil equivalent basis.

Labile P (NaCl P_i + NaCl P_o + $NaHCO_3$-P_i) and $NaHCO_3$ P_o were determined on finely ground air-dried soil using the same method as employed for sequential P fractionation previously described (4.2.1.1).

4.3.1.3 Statistical analysis

All statistical analysis were performed using R program version 2.15.0 (2012, The R Foundation for Statistical Computing) as described previously (4.2.1.3).

4.3.2 Results

Table 2.3.1 shows the concentrations of labile P, $NaHCO_3$-P_o and inorganic P released by phytase and alkaline phosphatase under *P. radiata*, *C. macrocarpa* and *E. nitens* for 0-5 cm soils sampled over 5 seasons. Degrees of freedom and associated *p* values can be seen in Table 4.3.2.

Table 4.3.2 shows the F values and levels of significance following a two way ANOVA for all the soil P forms under "species" and "season" and the interaction between "species" and "season". The analysis indicated that, except for the inorganic P released by alkaline phosphatase, where the concentrations were significantly higher under *E. nitens* (F=12.97; p<.001; *lsd* 5.3) there were no significant differences between species. Although there was not significant species-years interaction for inorganic P released by alkaline phosphatase, the greater release under *E. nitens* was mainly evident in the winter, spring and summer period (Table 4.3.1).

Table 4.3.1 Mean concentrations (mg/kg) of labile P, organic P in $NaHCO_3$ and inorganic P released by phytase and alkaline phosphatase under *P. radiata* (P.R.), *E. nitens* (E.N.) and *C. macrocarpa* (C.M.) in autumn-I, winter, spring, summer and autumn-II. Data in columns are means (n=4); data in parentheses are standard errors of the means.

Season	Labile P			$NaHCO_3$Po			Phytase			Alk. Phos.		
	P.R.	C.M.	E.N.	P.R.	C.M.	E.N.	P.R.	C.M.	E.N.	P.R.	C.M.	E.N.
						(mg/kg)						
Autumn-I	25.6	30.3	29.1	102	107.4	108.6	16.3	12.2	14.9	27.3	26.7	28.3
	(2.5)	*(2.5)*	*(2.2)*	*(6.1)*	*(8.0)*	*(4.5)*	*(2.4)*	*(3.3)*	*(1.6)*	*(1.8)*	*(4.2)*	*(2.7)*
Winter	22.8	29.9	25.8	121.7	132.3	124.9	24.3	22.0	23.2	34.6	33.0	48.7
	(2.3)	*(1.8)*	*(3.0)*	*(11.4)*	*(8.7)*	*(10.1)*	*(0.9)*	*(2.1)*	*(0.8)*	*(4.5)*	*(4.0)*	*(4.5)*
Spring	38.9	39	36.8	105.4	124.2	115.3	19.4	14.5	22.9	31.2	23.9	38.7
	(8.8)	*(6.6)*	*(3.0)*	*(10.3)*	*(8.0)*	*(5.9)*	*(4.4)*	*(4.4)*	*(1.4)*	*(3.1)*	*(3.8)*	*(2.9)*
Summer	24.8	30.5	32.2	97	99	97.4	8.8	9.2	13.7	18.3	19.1	33.1
	(1.8)	*(2.8)*	*(4.8)*	*(6.2)*	*(7.2)*	*(5.9)*	*(1.7)*	*(1.6)*	*(0.9)*	*(2.1)*	*(1.9)*	*(3.0)*
Autumn-II	25.7	26.6	29.9	85.4	103.2	121.7	13.5	15.7	17.8	26.8	29.0	30.7
	(1.3)	*(1.6)*	*(1.7)*	*(15.5)*	*(7.8)*	*(9.4)*	*(0.8)*	*(3.7)*	*(4.8)*	*(1.7)*	*(1.9)*	*(3.8)*

Table 4.3.2 F values and levels of significance from ANOVA for labile P, organic P in $NaHCO_3$ and inorganic P released by phytase and alkaline phosphatase for species, season and the interaction between species and season (Sp x S). Numbers in **bold** show significant differences (p<0.05).

	Source of variation					
	Species (Sp)		Season (S)		Sp x S	
Degrees of freedom→	2 df	P	4df	P	4df	P
Labile P	1.457	0.244	5.034	**0.002**	0.359	0.963
$NaHCO_3$ Po	2.758	0.174	5.015	**0.002**	0.856	0.560
Phytase Pi	2.481	0.095	9.352	**<0.001**	0.647	0.734
Alk. Phos. Pi	2.970	**<0.001**	9.327	**<0.001**	1.663	0.134

The effect of season was highly significant for all analyses, but there were no significant species x season interactions. Accordingly, data for enzyme labile $NaHCO_3$-extractable organic P determined under all tree species in each season is shown in Figure 4.3.1.

Results showed that inorganic P released was consistently higher for alkaline phosphatase (14-19 mg P/kg) compared with phytase (24-32 mg P/kg), reflecting the respective quantities of organic P hydrolysed by each enzyme. Alkaline phosphates hydrolysable P increased significantly from autumn to winter and then decreased during spring and summer before increasing again in the second autumn, while phytase hydrolysable P increased significantly from winter to spring but then declined to lower levels for the rest of the year (Fig. 4.3.1).

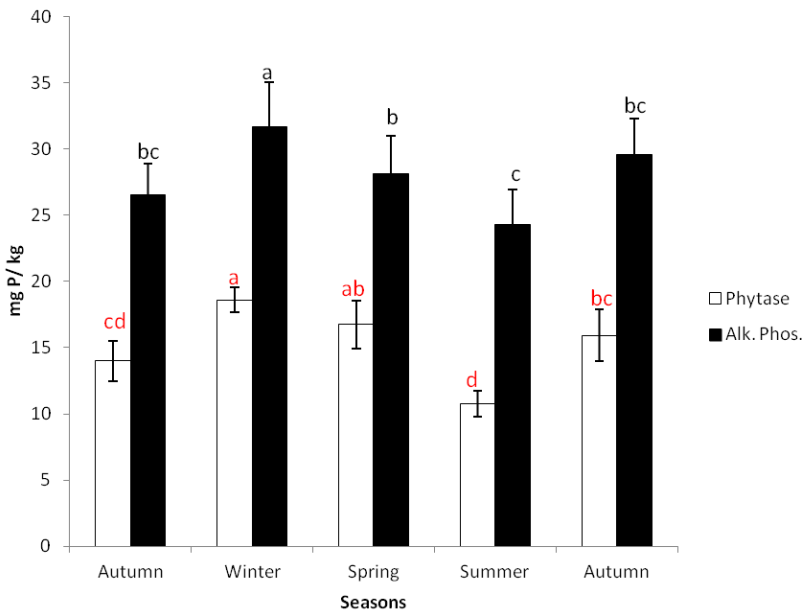

Figure 4.3.1 Mean concentrations of inorganic P (mg P/kg) released by phytase and alkaline phosphatase in each season under *P. radiata* for 0-5 cm soil. The error bars represent the standard errors of means. Different letters indicate that means were significantly different between sampling years ($p < 0.05$).

4.3.3. Discussion

It was hypothesised that seasonal variations in the quantities and nature of soil P would be influenced by tree species as it has been previously reported by other studies (Chen et al., 2003; Scott and Condron, 2003). However, results revealed that season was more important than species with respect to changes in soil P observed over time.

The results showed that the concentration of $NaHCO_3$ P_o and labile monoesters showed a seasonal trend of being highest in winter, and declining during spring and summer before increasing again in autumn.

Similar seasonal trends in soil P forms have been reported in other studies and have been attributed to a combination of factors; including root growth, uptake by plants, organic matter accumulation and microbial competition modifies the mineralization-immobilization processes (Chen et al., 2003; Fabre et al., 1996; McGrath et al., 2000; Perrott et al., 1990; Scott and Condron, 2003). For example, Chen et al. (2003) found that organic P was mineralized by increasing microbial activity to meet enhanced

plant demand in spring and summer, but accumulated during autumn and winter mainly due to slower plant growth and reduced microbial activity.

Seasonal changes in temperature and moisture are important in controlling soil microbial biomass and consequently the overall nutrient cycling rates and nutrient availability (Chen et al. 2003). Table 4.3.3 shows the mean monthly precipitation and temperature recorded for Banks Peninsula.

Table 4.3.3 Mean monthly precipitation and temperature recorded on Banks Peninsula (Metservice). Values in **bold** represent the months of seasonal sampling; dark highlights show the month with the highest values; pale highlights show the month with the lowest values.

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Precipitation (mm)	42	39	54	**54**	56	66	**79**	69	47	**53**	44	49
Temperature (°C)	**17.5**	17.2	15.5	**12.7**	9.8	7.1	**6.6**	7.9	10.3	**12.2**	14.1	16.1

He et al. (1997) conducted an experiment to investigate the response of the soil microbial biomass P to seasonal changes in the field over a 9-month period in the United Kingdom. They found that microbial biomass P is highly dependent on soil moisture, increasing during spring-summer (rainy season) and decreasing by autumn-winter. They also suggested that during spring and summer, the growth of plants would compete with the microbial biomass for soil P and consequently reduce P concentration in the soil solution. Similarly, Diaz-Ravina et al. (1995) found that microbial biomass C values were highest during spring and lower in summer, suggesting that moisture is a major factor controlling microbial population density and distribution.

Table 4.3.4 shows qualitative estimates for likely soil temperature, moisture, C inputs, organic P, labile P and consequent organic P mineralisation rate for each season at Orton Bradley Park. The most suitable conditions for microbial activity occur in spring and early summer, when temperature, moisture and organic C levels favour organic P mineralisation. On the other hand, although the moisture

conditions are high during winter, low soil temperature and reduced C inputs do not favor organic P mineralisation and may in fact promote immobilisation.

Table 4.3.4 Soil temperature and moisture, C inputs, concentrations of Po and labile P, and Po mineralisation rate expected to be found during each season.

	Temperature	Moisture	C inputs	[Po]	Labile P	Po mineralisation rate
Aut	√	x	High	Low	Low	Low
Win	x	√	Low	High	Low	Low
Spr	√	√	High	Intermediate	High	High
Sum	√	x	Low	Low	Low	Low

*√ favourable; ** x unfavourable

The release of P from biomass may also be attributed to fauna (e.g. protozoa) grazing on bacteria (Perrott et al., 1990). Also, root-feeding nematodes may increase the flow of carbon from roots to the soil microbial biomass, demonstrating the relationship between nematode functional groups and processes regulating decomposition processes (Yeates et al., 1997).

For this study, it was observed that enzyme lability and the increased mineralization of $NaHCO_3$ P_o was coupled with increased levels of labile P in spring, indicating a higher P demand by plants during this season. Scott and Condron (2003) conducted a field rhizosphere study over a period of 12 months to determine the concentrations of inorganic and organic P and phosphatase enzyme activity. They found lower levels of labile-Po coupled with higher levels of labile-Pi and enzyme activity in spring compared to autumn and winter. They attributed the decreased level of labile-Po to an increase in plant uptake suggesting that overall rates of soil organic P mineralisation are greatest in spring. This is similar to the trends reported in Table 4.3.4, indicating that higher levels of inorganic P are expected in spring due to an increase of mineralisation promoted by more suitable conditions for microbial communities.

Hayes et al. (2000) demonstrated that just a small component of the organic P in $NaHCO_3$ extracts can be hydrolysed by phytase, which is highly specific to *myo*-inositol hexakisphosphate and indicates that only low levels of potentially plant available organic P occur in soil solution. The results of this study support the previous work indicating that the release of inorganic P by phytase was low compared to alkaline phosphatase. Also, Turner et al. (2001) found that phytase activity appears to be inhibited in extracts of moist soils, which may contribute to the accumulation of inositol hexakisphosphate in soil. The increase of inositol hexakisphosphate in winter could be linked with accumulation of organic matter, as it has been reported by other studies who have shown that this form of organic P is mainly derived from plant residues (Celi and Barberis, 2005; Condron et al., 2005).

4.4 Conclusions

The findings of this study confirmed that significant net mineralisation of soil organic P occurred during the 5 years following tree planting, but that this was similar under the three contrasting tree species despite differences in growth and mycorrhizal association. The latter suggested that EM and AM may be equally effective at mineralizing soil organic P, and the observed changes in soil P could be attributed to tree growth and P uptake irrespective of species. However, reductions in P inputs and organic P turnover associated with the cessation of grazing may have also contributed to enhanced soil organic P mineralisation following forest establishment. In addition, changes in the nature of organic P determined between 0-5 and 5-10 years after establishment indicated that organic matter inputs and turnover associated with tree growth were having an increasing influence on soil P dynamics with time.

The findings of the seasonal study carried out when the trees were 12-13 years old confirmed that the bioavailability and mineralisation of soil organic P was greater in spring-summer compared with autumn-winter. This indicated that soil temperature was the main factor that influenced soil P dynamics (together with soil moisture), and surprisingly, the species of tree had no significant impact on seasonal changes in topsoil organic P.

CHAPTER 5 TEMPORAL CHANGES IN SOIL PHOSPHORUS UNDER ADJACENT GRASSLAND AND FOREST

5.1 Introduction

Results obtained from a wide range of paired-site comparison studies of grassland and adjacent plantation forest have consistently shown that afforestation of grassland can enhance net soil P mineralisation (Chen et al., 2008). However, most of these studies have been conducted at a single point in time, (commonly 10-20 years after forest establishment, approximately equivalent to 30-60% of rotation length) and do not provide details about the particular changes at different times during the rotation. Glendhu Forest in Otago provides an opportunity to study medium to long-term temporal changes in soil P under plantation forest compared with original grassland. Soil samples were initially collected from the grassland and adjacent forest in 1997, 15 years after planting, by Forest Research (SCION). The specific objective of the work described in this chapter was to re-sample the Glendhu grassland and forest sites, and assess and compare amounts and forms of soil P at the two different stages of forest development, namely 15 years (1997) and 28 years (2010) after establishment.

5.2 Materials and Methods

5.2.1 Glendhu

Adjoining catchments, one in grassland and one in *P. radiata* forest, form the Glendhu study site. The catchments are located on a north-facing hill slope in the Lammerlaw Range, eastern Otago, New Zealand (45 º 50’ S; 169 º 43’ E) (See Figure 5.1 and 5.2). The bedrock is quartzo-feldspathic schist (Fahey and Watson, 1991) and the soils are well drained Waipori silt loams which are Firm Brown Soils in the classification of Hewitt (1992) . Located at 460-670 m above sea level, the mean annual temperature is 14 °C and mean annual rainfall 1355 mm (Adams et al., 2001). The grassland catchment is dominated by snow tussock (*Chionochloa rigida*) and tall tussock grassland (*C. macra*) which occur in association with introduced grasses (Davis, 1994; Fahey and Watson, 1991). The catchments are used to study the long-term effects of converting tussock grassland to pine plantation. Both catchments were grazed prior to afforestation in 1982, and the grassland catchment is still lightly grazed by sheep. They have never been cultivated or fertilized. Soils were sampled in 1997 and 2010 along three replicated parallel transects selected at three elevations in each catchment area: approximately at 650 m, 620 m and 590 m elevation (Fig 5.1). At each elevation one transect was established in forest and one transect was established in the adjoining grassland. The transects at each site were similar in terms of aspect, slope and soil. The initial sampling was undertaken by Adams et al. (2001) and some general soil chemical properties determined at that sampling are shown in Table 5.1.

Figure 5.1 Satellite view of field research site at Glendhu Forest (source: Google Earth). The markers show the location of the transects at 650 (site 1), 620 (site 2) and 590 (site 3) m.

Figure 5.2 Forest development near the research site at Glendhu Forest. a) 1984, b) 1997 and c) in 2010 (photos provided by Barry Fahey).

Table 5.1 Acidity and ion exchange characteristics determined in soil under grassland and forest at 0-10 cm, 10-20 cm and 20-30 cm at Glendhu Forest (Adams *et al.*, 2001).

Depth (cm)	Site	% Soil moisture	pH	Na $CMl_c\ kg^{-1}$	K $CMl_c\ kg^{-1}$	Ca $CMl_c\ kg^{-1}$	Mg $CMl_c\ kg^{-1}$	KCl-Al $CMl_c\ kg^{-1}$	$CaCl_2$-Al $CMl_c\ kg^{-1}$
0-10	**Grassland**	52.9	4.7	0.16	0.55	0.93	0.89	11.6	0.31
	Forest	28.4	4.1	0.20	0.29	0.12	0.26	15.0	0.64
10-20	**Grassland**	35.6	4.8	0.10	0.29	0.27	0.42	12.6	0.38
	Forest	28.2	4.2	0.12	0.14	0.01	0.15	14.3	0.53
20-30	**Grassland**	29	4.8	0.04	0.14	0.10	0.16	11.2	0.36
	Forest	23.8	4.5	0.12	0.07	0.01	0.07	12.0	0.42

5.2.2 Soil Sampling and Analysis

Five cores of mineral soil were taken along each replicate transect, at intervals of 2 m at the three elevations in each catchment (tussock grassland and pine forest) at 3 depths (0-10, 10-20, 20-30 cm) in 1997 (15 years after planting) and 2010 (28 years after planting). Sampling at tussock bases was avoided for the grassland sites. For both, forest and grassland sites, overlying vegetation matter was removed to expose the mineral soil before sampling. The soil cores from each transect and depth were bulked together to give three replicate samples for each depth in each vegetation type. Equivalent soil samples taken at establishment (1987) were not available. Soil samples were air-dried at 30 °C, sieved (< 2 mm), finely ground and stored prior to soil P fractionation using the method described in Chapter 4 (Section 4.2.1.1).

5.2.3 Statistical Analysis

Statistical analyses were performed using R program (2012, The R Foundation for Statistical Computing). One and two way analyses of variance were performed to test the significance of time (1997, and 2010), vegetation treatment (grassland and

forest) and soil depth (0-10, 10-20 and 20-30 cm) on soil properties. Where F ratios were significantly different ($P< 0.05$) treatment means were compared by the Least Significant Differences (*lsd*) in a general ANOVA.

5.3 Results

Table 5.2 shows data for concentrations of labile P, $NaHCO_3$ P_o, NaOH-I P_i, NaOH-I P_o, HCl P_i, NaOH-II P_i, NaOH-II P_o, total extractable P_i (ΣP_i) and total extractable P_o (ΣP_o) determined for each soil depth (0-10, 10-20 and 20-30 cm) under both grassland and forest sites in 1997 and 2010. All soil P fractions decreased with depth under grassland and forest at both sampling dates.

On average, the labile P, NaOH-I P_i, HCl P_i and NaOH-II P_i fractions represented 19, 62, 4 and 15% of total extractable inorganic P, respectively, while the $NaHCO_3$ P_o, NaOH-I P_o and NaOH-II P_o fractions represented 16, 62 and 22% of total extractable organic P, respectively. Data presented in Figures 5.3 and 5.4 show that labile P, NaOH-I P_i and total extractable inorganic P were significantly higher under forest compared with grassland for all soil depths in 1997 and 2010. Concentrations of HCl P_i in 1997 and NaOH-II P_i in 2010 were also slightly higher under forest at all soil depths. Conversely, concentrations of organic P in the $NaHCO_3$, NaOH-I and total extractable pools were significantly lower for all soil depths under forest compared with grassland in 1997 and 2010. It is also clear from this data that similar concentrations of P were determined in the various soil fractions under grassland in 1997 and 2010. In soils under forest the concentrations of labile P and HCl P_i decreased significantly for all soil depths between 1997 and 2010, while NaOH-II P_i increased significantly in the 10-20 and 20-30 cm layers between 1997 and 2010 (Figure 5.3, 5.5). As a consequence, the overall decrease in total extractable inorganic P between 1997 and 2010 was only significant for the 20-30 cm soil.

Table 5.2 Mean concentrations (mg P/ kg) of labile P, $NaHCO_3$ P_o, NaOH-I P_i, NaOH-I P_o, HCl P_i, NaOH-II P_i, NaOH-II P_o and total extractable P_i and P_o fractions under grassland and forest at 3 depths (0-10, 10-20 and 20-30 cm) in 1997 and 2010. Data in columns are means (n=3); data in parenthesis are standard errors of means.

	Depth	Year	Labile P	$NaHCO_3$ Po	NaOH-I P_i	NaOH-I P_o	HCl P_i	NaOH-II P_i	NaOH-II P_o	ΣP_i	ΣP_o
			(mg P/ kg)								
Grassland	0-10	1997	30.7	81.5	96.6	318.7	7.5	27.9	103.8	162.7	503.9
			(0.6)	*(1.7)*	*(4.8)*	*(3.9)*	*(0.3)*	*(0.7)*	*(8.6)*	*(4.6)*	*(6.4)*
		2010	33.4	81.7	102.8	316.3	8.5	28.8	98.8	173.5	496.9
			(0.6)	*(1.2)*	*(2.6)*	*(2.6)*	*(0.3)*	*(0.8)*	*(6.2)*	*(2.4)*	*(5.2)*
	10-20	1997	27.2	79.4	89.5	301.8	6.9	24.0	92.9	147.5	474.2
			(1.5)	*(0.4)*	*(6.4)*	*(0.7)*	*(0.3)*	*(1.1)*	*(12.2)*	*(8.9)*	*(11.0)*
		2010	30.0	79.7	91.3	308.0	7.7	24.9	91.1	153.9	478.8
			(1.5)	*(0.5)*	*(5.3)*	*(1.3)*	*(0.2)*	*(1.1)*	*(11.3)*	*(6.4)*	*(11.4)*
	20-30	1997	21.2	75.5	73.4	293.3	6.4	18.8	81.5	119.7	450.3
			(1.0)	*(2.4)*	*(3.3)*	*(14.4)*	*(0.6)*	*(2.6)*	*(12.8)*	*(5.9)*	*(26.9)*
		2010	24.0	75.7	72.7	291.2	5.9	19.7	79.6	122.3	446.6
			(0.9)	*(2.4)*	*(3.6)*	*(10.1)*	*(1.1)*	*(2.6)*	*(12.8)*	*(4.3)*	*(21.2)*
Forest	0-10	1997	54.8	62.2	179.8	233.2	11.5	31.3	101.2	277.4	396.6
			(2.5)	*(2.4)*	*(9.3)*	*(10.6)*	*(0.2)*	*(7.4)*	*(6.5)*	*(18.1)*	*(4.8)*
		2010	44.9	63.0	155.7	225.8	7.4	48.5	106.7	256.5	395.5
			(2.5)	*(1.2)*	*(9.1)*	*(10.6)*	*(0.2)*	*(7.0)*	*(9.4)*	*(18.1)*	*(2.2)*
	10-20	1997	49.3	61.3	164.1	220.7	11.0	23.4	96.3	247.8	378.3
			(1.5)	*(2.2)*	*(13.5)*	*(15.4)*	*(0.2)*	*(2.7)*	*(9.6)*	*(17)*	*(6.8)*
		2010	41.7	59.6	139.9	213.3	6.9	40.7	98.5	229.2	371.4
			(1.7)	*(3.4)*	*(13.5)*	*(15.4)*	*(0.2)*	*(2.7)*	*(9.6)*	*(17.7)*	*(6.9)*
	20-30	1997	44.3	47.2	145.8	202.6	10.7	16.7	79.0	217.5	328.9
			(0.9)	*(2.0)*	*(12.4)*	*(13.6)*	*(0.4)*	*(1.7)*	*(2.3)*	*(12.1)*	*(15.5)*
		2010	35.6	46.8	121.6	195.3	6.6	34.0	81.2	197.8	323.3
			(1.0)	*(1.9)*	*(12.1)*	*(14)*	*(0.4)*	*(1.6)*	*(2.5)*	*(12.4)*	*(15.2)*

*Labile P is the sum of P_i in NaCl + inorganic P in $NaHCO_3$; **ΣP_i is the sum of inorganic P in NaCl, $NaHCO_3$, NaOH-I, HCl, and NaOH-II; ***ΣP_o is the sum of organic P in NaCl, $NaHCO_3$, NaOH-I and NaOH-II.

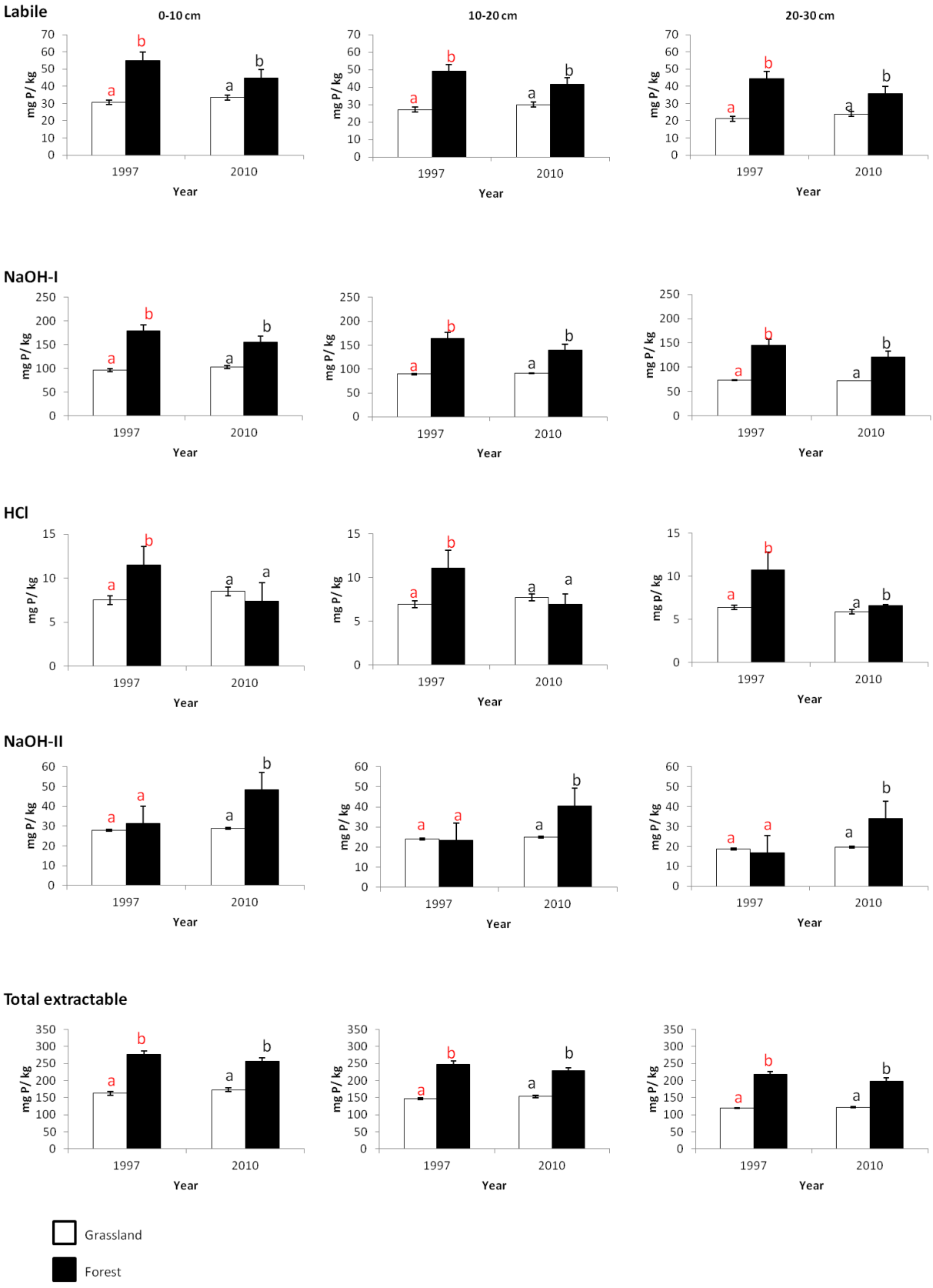

Figure 5.3 Mean concentrations of inorganic P (mg P/kg) in labile, NaOH-I, HCl, NaOH-II and total extractable fractions in 0-10, 10-20 and 20-30 cm soils depths sampled under grassland and forest in 1997 and 2010. The error bars represent standard errors of means. Different letters indicate that means were significantly different between grassland and forest within each year ($p < 0.05$).

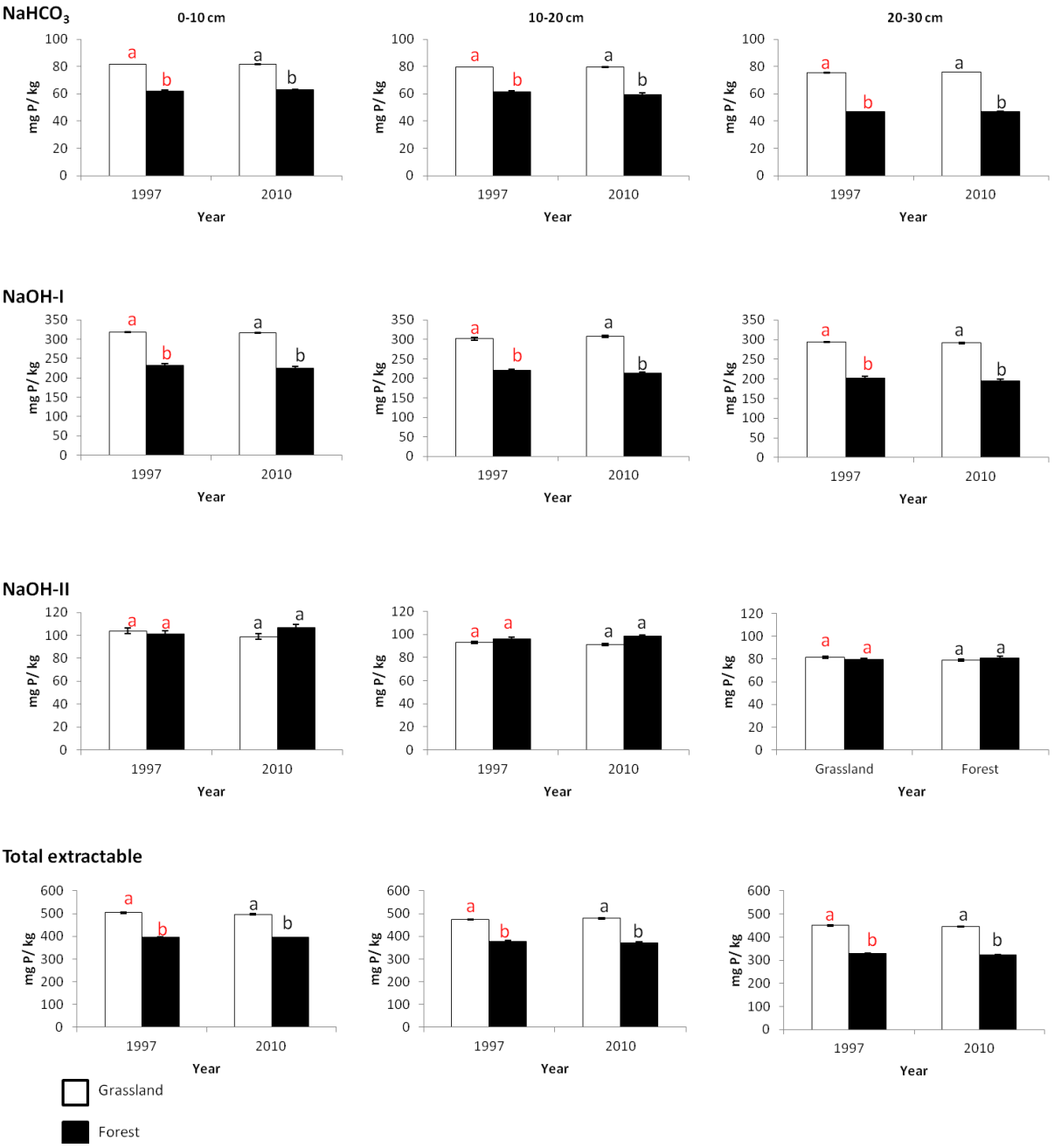

Figure 5.4 Mean concentrations of organic P (mg P/kg) in $NaHCO_3$, NaOH-I, NaOH-II and total extractable fractions in 0-10, 10-20 and 20-30 cm soils depths sampled under grassland and forest in 1997 and 2010. The error bars represent standard errors of means. Different letters indicate that means were significantly different between grassland and forest within each year ($p < 0.05$).

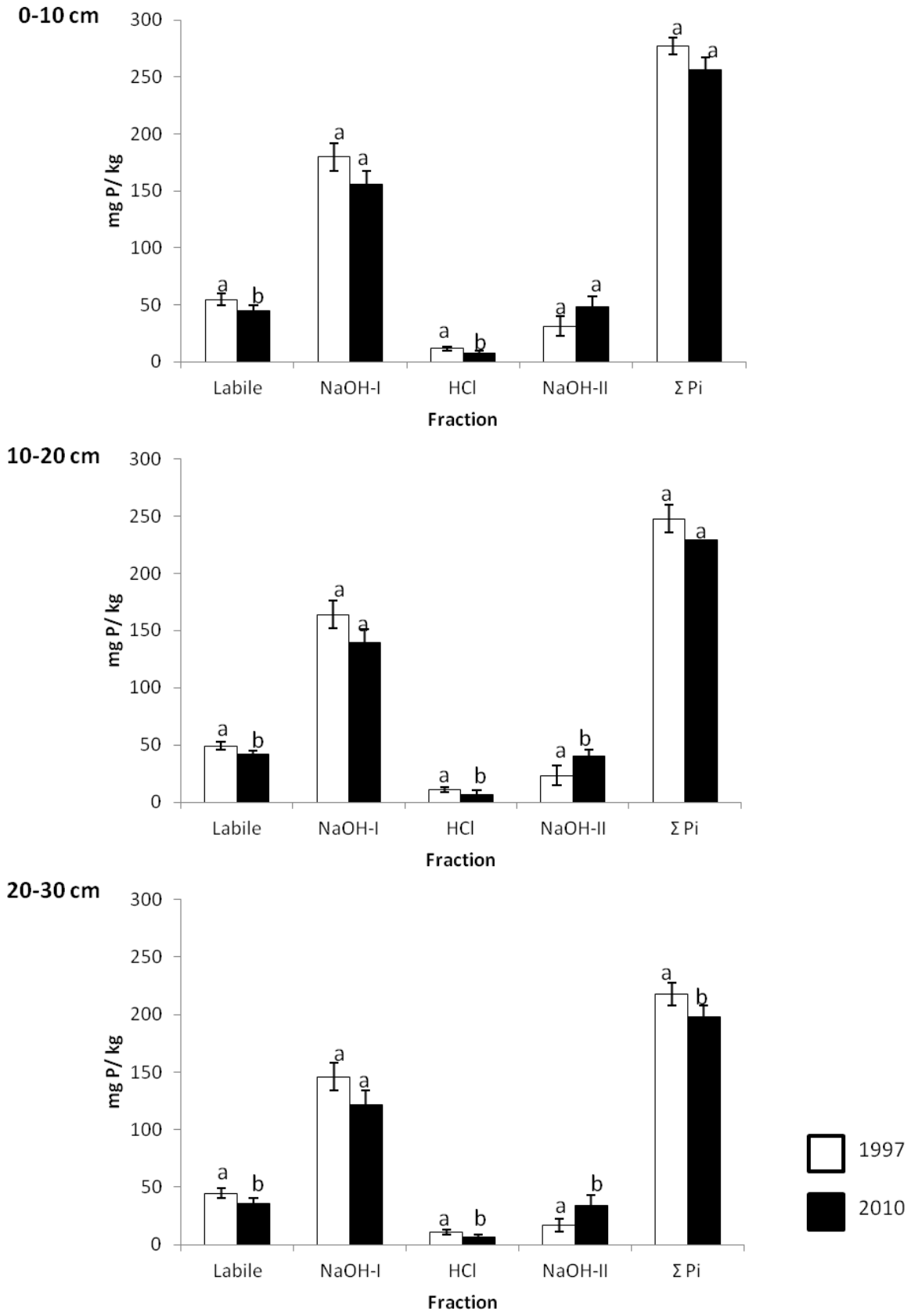

Figure 5.5 Mean concentrations of inorganic P (mg P/kg) for 0-10 cm, 10-20 cm and 20-30 cm soils depths sampled under forest in 1997 and 2010. The error bars represent standard errors of means. Different letters indicate that means were significantly different between sampling years ($p < 0.05$).

5.4 Discussion

The significant decreases in soil organic P and concomitant increases in inorganic P that were evident under forest compared with grassland 15 years after establishment indicate that enhanced mineralisation of organic P occurred as consequence of the change in land-use at Glendhu. This is consistent with similar findings from a wide range of one-time paired-site comparative studies of soil P changes under adjacent grassland and forest areas(Chen et al., 2008). However, similarities in the relative magnitude of differences in the various soil inorganic and organic P fractions measured between grassland and forest soils in 1997 and 2010 were unexpected. Davis and Condron (2002) collated and analysed data on the impacts of afforestation on soil organic C in New Zealand. Results revealed that after 10-15 years organic C was significantly lower under forest compared with adjacent grassland by an average of 10%, although corresponding differences between grassland and forest were smaller and not significant for older forests. These findings suggest that as forest development proceeds through the first rotation organic matter inputs from trees increase with time leading to accumulation of organic C in soil after the initial reduction, although the nature and dynamics of the forest soil organic C is likely to be different and more recalcitrant than under grassland (Condron and Newman, 1998). It was expected that the relative magnitude of differences in soil P fractions between grassland and forest would also change with time as the forest matured. The fact that this did not occur at Glendhu suggests that changes in the quantity and form of soil inorganic and organic P associated with afforestation occurred during the first 15 years following forest establishment. This in turn is consistent with findings from the Orton Bradley Park field trial reported in Chapter 2 of this thesis.

The fact that the amounts and fractional distribution of soil inorganic and organic P were similar under grassland sampled in 1997 and 2010 was not surprising as the productivity of the unfertilised extensive pasture system at Glendhu was very low. However, significant changes in the soil inorganic P fractions under forest between 1997 and 2010 could be related to continued P uptake by trees over this period.

Thus the significant decreases in labile P and HCl P_i, together with consistent but non-significant decrease in NaOH-I P_i determined in soils under forest between 1997 and 2010 may be mainly attributed to P uptake by trees and storage in biomass. There was no forest biomass data available for Glendhu, but it is reasonable to assume that significant biomass expansion and consequent P acquisition occurred between 1997 and 2010 (see Fig. 5.2). The fact that NaOH-II P_i increased significantly in 10-30cm soils under forest between 1997 and 2010 indicates a shift towards more recalcitrant soil P_i forms with continued forest development, which in turn might be linked to a decrease in soil pH (Table 5.3).

Table 5.3 Soil pH (0-10 cm) determined under grassland and forest in 1997 and 2010 (standard errors of means are shown in parenthesis).

	Forest	Grassland
1997	4.3	4.8
	(0.13)	(0.09)
2010	4.1	4.9
	(0.10)	(0.07)

5.5 Conclusions

The findings of this study confirmed that afforestation of grazed grassland significantly enhanced mineralisation of soil organic P resulting in concomitant increases in soil inorganic P. The fact that differences in soil P between adjacent grassland and forest soils were mostly similar in 1997 and 2010 (15 and 28 years after forest establishment, respectively), indicates that changes in soil P under trees compared with grassland occurred mainly during the early stages of forest development. However, an increase in soil NaOH-II extractable inorganic P after 1997 indicated that P may be becoming more recalcitrant as forest age increased. While amounts and forms of soil P under grassland were similar at both sampling times, continued P uptake by trees resulted in a significant depletion of soil inorganic P reserves, except for more recalcitrant inorganic P, between 1997 and 2010.

CHAPTER 6 CHANGES IN SOIL PHOSPHORUS ASSOCIATED WITH ESTABLISHMENT AND DEVELOPMENT OF A SILVOPASTORAL SYSTEM

6.1 Introduction

Silvopastoral systems in New Zealand combine the growing of trees (commonly *P. radiata*) with grazed pasture (e.g. *Lolium perenne* and *Trifolium repens*), and constitute a small part of the expansion of short-rotation plantation forestry that occurred since the early 1980's. In conventional *P. radiata* forest the common practice is to plant 1000-1200 stems per hectare (sph) and to thin this to a final crop of 250-350 sph within 6-8 years; the trees are also pruned 3-4 times over this period to enable a minimum 6 m butt-log to be produced at harvest (25-35 years). In silvopastoral systems the initial planting density is less than conventional forestry (800-1000 sph) and the aim is to establish fewer (e.g. 150-200 sph), more highly pruned trees (e.g. 8-10 m), in wider spaced rows to prevent canopy closure and thereby enable persistence of productive pasture. The latter also requires that thinned trees and debris from pruning operations are removed, while in conventional forests these are left to decompose.

A silvopastoral field trial was established adjacent to Lincoln University in 1990 on land that had been farmed for many years under an intensive crop-pasture rotation system. The main objective of the trial was to investigate the effects of different *P. radiata* clones and combinations of understory pasture species on tree growth, pasture performance and associated nutrient and soil water dynamics (see below). The trial was managed for 10 years until 2000 when the loss of continued funding meant that maintenance of the trial was terminated. Figure 4.2 shows the status of the trial in 2000 with well-established trees and understory pasture. Over the following years continued tree growth in the absence of any further thinning or pruning lead to gradual canopy closure with an associate decline in pasture productivity. By 2010 there was little or no evidence of any pasture understory and the site resembled a conventional plantation forest (Figure 6.8).

Soil samples were collected at experiment establishment in 1991 and again in 2000 and 2010. The Lincoln silvopastoral field trial therefore offers a unique opportunity to assess the impact of simultaneous tree and pasture establishment on the amounts, forms and bioavailability of soil P. Accordingly, the objective of this study was to investigate and quantify changes in soil P that occurred over the 20 year duration of the silvopastoral trial by comparing data for soil samples collected in 1991, 2000 and 2010.

6.2 Materials and methods

6.2.1 Lincoln University Silvopastoral Field Trial

The Silvopastoral Field Trial is located two km northwest of Lincoln University (43 º 37' S; 172 º 26' E) on fertile free draining arable soil (soil classification: Templeton silt loam or podzolised yellow brown earth), on one to two meters of fine alluvial sediments underlain by stones and gravels, with a moderate water holding capacity. The climate is temperate and subhumid with an annual precipitation of 660 mm and a mean annual temperature of 11.4 ºC. Between May and September mean monthly temperatures usually fall below 10 ºC and ground frosts are common (Mead *et al*., 2010).

The field trial experiment was established in 1990 to evaluate the competitive effects of different agricultural pasture understorey species on radiata pine over time. It consisted of six treatments replicated three times in a randomized block design. The treatments were: bareground, phalaris (*Phalaris arundinacea*); cocksfoot-clover (*Dactylis glomerata – Trifolium repens*); ryegrass-clover (*Lolium perenne – Trifolium repens*); lucerne (*Medicago sativa*) and ryegrass alone (See Figure 6.1). No fertilizers were applied since establishment and rotational grazing by sheep occurred during the first 10 years. The area of each plot was 0.194 ha and the trees were planted within the plot at 7 m by 1.4 m spacing (1000 stems ha^{-1}). Trees were thinned in 1992, 1993 and 1994, and were pruned annually between 1994 until the trial was abandoned in 2000 (Mead *et al*., 2010).

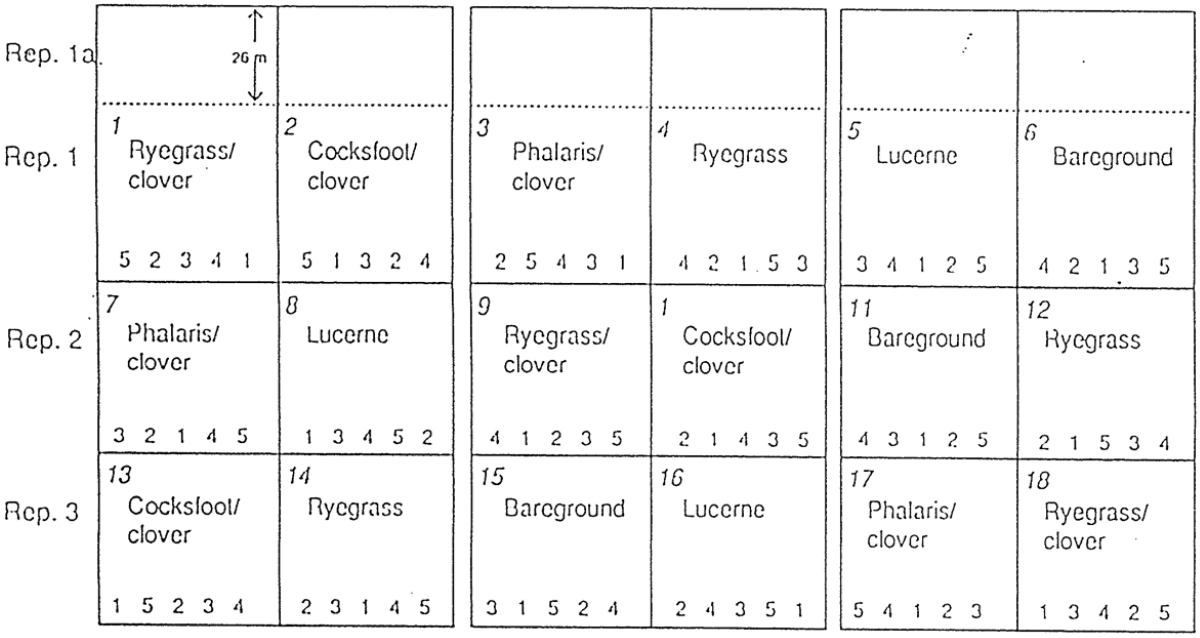

Figure 6.1 The randomized plot design of the silvopastoral trial site at Lincoln University Silvopastoral Field.

6.2.2 Soil Sampling and Analysis

Three out of the six original understory treatments were selected for this study: ryegrass-clover, cocksfoot-clover and lucerne. Following a sampling protocol developed in 1991, 15 cores of mineral soil were collected adjacent to the tree rows and bulked from 4 depths (0-7.5, 7.5-15, 15-22.5, 22.5-30 cm) from each of the 3 replicate plots at the three selected understorey treatments (9 plots) in 1991 (at trial establishment), 2000 (9 years after establishment) and 2010 (19 years after establishment). Soil samples were air-dried and sieved to <2 mm prior to soil analysis.

The sequential fractionation method (previously described in Chapter 4) was used to determine the different forms of inorganic and organic P.

Concentrations of $NaHCO_3$ extractable organic P mineralized by alkaline phosphatase and phytase were determined in 0-7.5 cm soil samples collected in 1991, 2000 and 2010 under lucerne (3 replicates per year).

6.2.3 Statistical Analysis

Statistical analyses were performed using R program (2012, The R Foundation for Statistical Computing). One and two way analyses of variance were carried out on the data to test significant of effects on the variables, year (1991, 2000 and 2010), treatment (ryegrass-clover, cocksfoot-clover and lucerne) and depth (0-7.5, 7.5-15, 15-22.5, 22.5-30 cm) on soil properties. Where F ratios were significant ($P< 0.05$) treatment means were compared by the Least Significant Differences (*lsd*) test in a general ANOVA.

6.3 Results

6.3.1 Soil P Fractionation

Table 6.1 shows data for concentrations of labile P, $NaHCO_3$ P_o, NaOH-I P_i, NaOH-I P_o, HCl P_i, NaOH-II P_i, NaOH-II P_o, total extractable P_i and P_o, determined under ryegrass-clover, cocksfoot-clover and lucerne for each depth (0-7.5, 7.5-15, 15-22.5 and 22.5-30 cm) in 1991, 2000 and 2010. Levels of significance are shown in Table 6.2.

On average, the labile P, NaOH-I P_i, HCl P_i and NaOH-II P_i fractions represented 10, 50, 27 and 13% of total extractable inorganic P, respectively, while the $NaHCO_3$ P_o, NaOH-I P_o and NaOH-II P_o fractions represented 21, 71 and 8% of total extractable organic P, respectively.

Results showed that labile P, NaOH-I P_i, HCl P_i and NaOH-II P_i consistently decreased with depth under most of the understories, except for NaOH-I P_i under cocksfoot-clover and HCl P_i under ryegrass-clover.

Concentrations of $NaHCO_3$ P_o were consistently greater in 0-22.5 cm soils than 22.5-30 cm soils. Although it was evident that the NaOH-I P_o concentrations were higher in the topsoil, no consistent statistical differences were observed between depths, understories and years, while NaOH-II P_o was slightly higher in the top 15 cm than 15-30 cm.

Table 6.1 Mean P concentrations (mg P/ kg) of labile P, $NaHCO_3$ P_o, NaOH-I P_i, NaOH-I P_o, HCl P_i, NaOH-II P_i, NaOH-II P_o and total extractable P_i and P_o fractions under ryegrass-clover and cocksfoot-clover at 4 depths (0-7.5, 7.5-15, 15-22.5, 22.5-30 cm) in 1991, 2000 and 2010. Data in columns are means (n=4); data in parenthesis are standard errors of means.

	Depth	Year	Labile P	$NaHCO_3$ P_o	NaOH-I P_i	NaOH-I P_o	HCl P_i	NaOH-II P_i	NaOH-II P_o	ΣP_i	ΣP_o	ΣP
						(mg/kg)						
Ryegrass-clover	0-7.5											
		1991	60.0	59.1	196.6	273.3	84.5	45.9	35.1	387	367.5	754.5
			(6.0)	*(15.7)*	*(23)*	*(13.2)*	*(14.0)*	*(1.7)*	*(4.5)*	*(11.0)*	*(27.4)*	*(38.4)*
		2000	31.1	88.8	157.4	257.7	94.2	40.1	49.0	322.8	395.5	718.3
			(1.4)	*(6.3)*	*(28.7)*	*(29.0)*	*(11.1)*	*(1.5)*	*(10.1)*	*(31.7)*	*(37.9)*	*(69.6)*
		2010	38.6	106.7	182.4	261.3	94.9	41.0	24.9	356.9	392.9	749.8
			(3.8)	*(9.2)*	*(11.1)*	*(7.1)*	*(13.7)*	*(3.6)*	*(2.4)*	*(19.1)*	*(15.5)*	*(34.6)*
	7.5-15											
		1991	39.9	60.4	196.9	275.6	90.4	47.2	21.5	374.4	357.5	731.9
			(2.3)	*(17.1)*	*(20.5)*	*(39.4)*	*(10.9)*	*(2.2)*	*(3.6)*	*(18.0)*	*(59.3)*	*(77.3)*
		2000	31.3	93.9	141.2	254.8	90.2	39.1	47.0	301.8	395.7	697.5
			(1.2)	*(2.5)*	*(21.1)*	*(21.3)*	*(12.2)*	*(2.0)*	*(10.3)*	*(25.7)*	*(29.3)*	*(55.0)*
		2010	30.2	98.7	162.3	275.3	91.9	39.0	34.4	323.4	408.4	731.8
			(0.6)	*(0.3)*	*(11.4)*	*(10.6)*	*(13.4)*	*(2.4)*	*(3.1)*	*(22.1)*	*(12.7)*	*(34.8)*
	15-22.5											
		1991	30.5	51.3	161.8	248.3	75.3	44.6	21.8	312.2	321.4	633.6
			(2.0)	*(13.9)*	*(9.7)*	*(23.3)*	*(10.0)*	*(0.8)*	*(1.5)*	*(10.9)*	*(37.7)*	*(48.6)*
		2000	22.8	80.7	122.8	234.2	83.8	36.6	43.1	266	358	624.0
			(0.9)	*(10.9)*	*(21.7)*	*(23.4)*	*(12.0)*	*(1.3)*	*(9.8)*	*(30.4)*	*(28.2)*	*(58.6)*
		2010	25.0	82.1	139.3	248.7	86.3	36.7	19.4	287.3	350.2	637.5
			(0.6)	*(1.5)*	*(12.3)*	*(16.9)*	*(11.2)*	*(2.3)*	*(4.7)*	*(21.9)*	*(20.3)*	*(42.2)*
	22.5-30	1991	21.9	29.7	122.4	172.7	54.8	39.8	23.1	238.9	225.5	464.4
			(3.7)	*(7.0)*	*(2.3)*	*(19.0)*	*(6.3)*	*(2.1)*	*(2.1)*	*(9.9)*	*(27.7)*	*(37.6)*
		2000	12.1	53.1	95.0	198.1	67.2	32.8	25.3	207.1	276.5	483.6
			(0.7)	*(17.8)*	*(17.9)*	*(25.9)*	*(11.1)*	*(2.3)*	*(4.5)*	*(28.4)*	*(24.5)*	*(52.9)*
		2010	13.8	50.6	105.4	145.9	65.2	35.7	18.8	220.1	215.3	435.4
			(0.8)	*(2.5)*	*(10.2)*	*(3.5)*	*(9.1)*	*(2.4)*	*(3.5)*	*(18.3)*	*(8.8)*	*(27.1)*
Cocksfoot-clover												
	0-7.5											
		1991	60.0	60.3	216.0	244.5	89.1	47.5	29.3	412.6	334.1	746.7
			(8.1)	*(15.4)*	*(11.5)*	*(12.0)*	*(18.5)*	*(1.1)*	*(2.1)*	*(37.5)*	*(28.6)*	*(66.1)*
		2000	35.6	94.7	159.7	258.6	99.0	39.1	43.2	333.4	396.5	729.9
			(5.1)	*(5.8)*	*(16.8)*	*(14.6)*	*(12.5)*	*(1.9)*	*(5.3)*	*(20.6)*	*(18.0)*	*(38.6)*
		2010	42.3	102.9	190.7	252.4	95.8	37.4	29.4	366.2	384.7	750.9
			(1.9)	*(6.3)*	*(4.4)*	*(10.8)*	*(14.0)*	*(2.0)*	*(8.0)*	*(17.9)*	*(11.6)*	*(29.5)*
	7.5-15											
		1991	44.1	62.0	224.8	252.3	89.5	49.7	28.0	408.1	342.3	750.4
			(9.1)	*(16.2)*	*(17.6)*	*(16.8)*	*(14.4)*	*(2.2)*	*(5.0)*	*(39.0)*	*(27.6)*	*(66.6)*
		2000	32.3	97.1	132.8	261.5	91.1	37.2	37.3	293.4	395.9	689.3
			(1.4)	*(1.8)*	*(14.3)*	*(21.7)*	*(11.9)*	*(1.0)*	*(0.0)*	*(18.6)*	*(23.1)*	*(41.7)*
		2010	33.2	93.5	172.2	242.3	94.8	38.0	25.3	338.2	361.1	699.3
			(1.1)	*(3.0)*	*(10.7)*	*(17.8)*	*(15.6)*	*(4.6)*	*(3.0)*	*(25.0)*	*(17.6)*	*(42.6)*
	15-22.5											
		1991	32.3	53.6	203.8	242.2	76.2	45.9	25.1	358.2	320.9	679.1
			(5.9)	*(14.6)*	*(18.6)*	*(7.4)*	*(15.3)*	*(1.7)*	*(1.4)*	*(39)*	*(23.1)*	*(62.1)*
		2000	27.4	83.1	125.7	239.8	86.6	36.6	29.9	276.3	352.8	629.1
			(3.3)	*(8.3)*	*(17.4)*	*(17.3)*	*(13.5)*	*(1.4)*	*(2.6)*	*(26.1)*	*(21.8)*	*(47.9)*
		2010	24.1	78.5	141.8	263.9	86.1	35.1	24.5	287.1	366.9	654.0
			(4.3)	*(9.1)*	*(9.6)*	*(5.1)*	*(13.7)*	*(4.2)*	*(3.5)*	*(22.3)*	*(1.2)*	*(23.5)*
	22.5-30	1991	20.2	31.5	152.3	178.1	52.2	37.9	18.5	262.6	228.1	490.7
			(3.7)	*(7.0)*	*(2.3)*	*(19.0)*	*(6.3)*	*(2.1)*	*(2.3)*	*(9.9)*	*(27.7)*	*(37.6)*
		2000	15.3	41.8	94.7	186.5	68.0	31.8	27.9	209.8	256.2	466.0
			(2.1)	*(6.1)*	*(11.2)*	*(12.5)*	*(15.6)*	*(2.5)*	*(2.2)*	*(28.3)*	*(19.5)*	*(47.8)*
		2010	14.7	46.9	103.8	210.1	67.6	32.1	25.0	218.2	282	500.2
			(2.0)	*(2.5)*	*(12.4)*	*(12.6)*	*(15.0)*	*(3.1)*	*(5.4)*	*(26.6)*	*(19.0)*	*(45.6)*

*Labile P is the sum of inorganic and organic P in NaCl; **ΣP_i is the sum of inorganic P in NaCl, $NaHCO_3$, NaOH-I, HCl, and NaOH-II; ***ΣP_o is the sum of organic P in NaCl, $NaHCO_3$, NaOH-I and NaOH-II; ****ΣP is the sum of ΣP_i and ΣP_o.

Table 6.1 (continued) Mean P concentrations (mg P/ kg) of labile P, $NaHCO_3$ P_o, NaOH-I P_i, NaOH-I P_o, HCl P_i, NaOH-II P_i, NaOH-II P_o and total extractable P_i and P_o fractions under lucerne at 4 depths (0-7.5, 7.5-15, 15-22.5, 22.5-30 cm) in 1991, 2000 and 2010. Data in columns are means (n=4); data in parenthesis are standard errors of means.

	Depth	Year	Labile P	$NaHCO_3$ P_o	NaOH-I P_i	NaOH-I P_o	HCl P_i	NaOH-II P_i	NaOH-II P_o	ΣP_i	ΣP_o	ΣP
						(mg/kg)						
Lucerne	0-7.5											
		1991	49.8	64.2	188.7	268.1	89.7	45.6	25.7	373.8	358	731.8
			(8.9)	*(17.8)*	*(19.6)*	*(5.7)*	*(11.6)*	*(1.1)*	*(4.6)*	*(7.3)*	*(22.1)*	*(29.4)*
		2000	30.0	89.1	149.3	273.5	95.4	38.5	45.1	313.2	407.7	720.9
			(2.4)	*(7.6)*	*(11.2)*	*(17.0)*	*(7.4)*	*(3.0)*	*(5.4)*	*(4.2)*	*(19.3)*	*(23.5)*
		2010	33.8	104.0	169.3	302.6	89.8	39.0	27.5	331.9	434.1	766.0
			(0.5)	*(4.5)*	*(2.8)*	*(14.9)*	*(4.6)*	*(2.6)*	*(3.5)*	*(0.8)*	*(20.4)*	*(21.2)*
	7.5-15											
		1991	35.6	62.9	188.4	281.5	84.6	44.9	23.1	353.5	367.5	721.0
			(5.9)	*(17.0)*	*(22.5)*	*(4.1)*	*(6.3)*	*(2.9)*	*(1.7)*	*(22.8)*	*(19.0)*	*(41.8)*
		2000	29.9	91.7	129.0	267.8	94.0	36.6	37.3	289.5	396.8	686.3
			(2.1)	*(5.3)*	*(9.6)*	*(29.6)*	*(6.0)*	*(2.6)*	*(5.4)*	*(3.3)*	*(29.5)*	*(32.8)*
		2010	26.3	95.7	145.0	288.2	90.2	41.0	29.3	302.5	413.2	715.7
			(1.1)	*(2.6)*	*(3.1)*	*(14.9)*	*(5.8)*	*(3.5)*	*(1.3)*	*(6.5)*	*(15.9)*	*(22.4)*
	15-22.5											
		1991	30.6	52.7	167.7	262.3	77.8	42.7	24.1	318.8	339.1	657.9
			(4.8)	*(13.4)*	*(14.5)*	*(14.0)*	*(9.9)*	*(2.8)*	*(2.6)*	*(12.3)*	*(17.5)*	*(29.8)*
		2000	23.5	74.8	120.9	258.7	86.4	36.0	32.8	266.8	366.3	633.1
			(0.5)	*(4.5)*	*(8.9)*	*(27.0)*	*(5.5)*	*(2.2)*	*(5.5)*	*(2.3)*	*(23.3)*	*(25.6)*
		2010	21.6	81.6	130.3	287.9	82.2	40.0	22.4	274.1	391.9	666.0
			(0.8)	*(6.3)*	*(4.2)*	*(20.0)*	*(4.2)*	*(3.3)*	*(1.8)*	*(5.5)*	*(26.3)*	*(31.8)*
	22.5-30	1991	20.8	26.4	131.6	229.1	54.2	40.2	22.1	246.8	277.6	524.4
			(2.5)	*(5.7)*	*(12.9)*	*(21.2)*	*(8.7)*	*(3.1)*	*(2.2)*	*(18.3)*	*(27.5)*	*(45.8)*
		2000	13.1	38.2	103.4	217.4	69.7	33.7	27.9	219.9	283.5	503.4
			(1.1)	*(2.7)*	*(10.2)*	*(23.0)*	*(8.7)*	*(2.8)*	*(2.6)*	*(16.5)*	*(21.9)*	*(38.4)*
		2010	12.3	48.6	93.9	239.1	54.7	37.1	18.2	198	305.9	503.9
			(0.5)	*(2.4)*	*(1.1)*	*(11.2)*	*(3.2)*	*(3.1)*	*(3.3)*	*(1.3)*	*(8.3)*	*(9.6)*

*Labile P is the sum of inorganic and organic P in NaCl; **ΣP_i is the sum of inorganic P in NaCl, $NaHCO_3$, NaOH-I, HCl, and NaOH-II; ***ΣP_o is the sum of organic P in NaCl, $NaHCO_3$, NaOH-I and NaOH-II; ****ΣP is the sum of ΣP_i and ΣP_o.

Table 6.2 shows the F values and their levels of significance following two way ANOVA for all the soil fractions for “understorey treatments” and “year” (1991, 2000 and 2010) and the interaction between “understorey” and “year”. The analyses indicated that except for NaOH-I P_o at 22.5-30 cm, where the concentrations were significantly higher under lucerne ($F= 3.54$; $p < 0.05$), there were no significant differences due to understorey treatment and no significant year by understorey interactions.

Table 6.2 F values levels of significance from an ANOVA for all the soil P fractions under ryegrass-clover, cocksfoot-clover and lucerne in 1991, 2000 and 2010 and the interaction between understorey and year (U x Y) for 0-7.5, 7.5-15, 15-22.5 and 22.5-30 cm soil depths. Numbers in **bold** show significant differences (p <0.05).

		Source of variation					
		Understorey (U)		Year (Y)		U x Y	
	Degrees of freedom→	F	*p*	F	*p*	F	*p*
0-7.5	Labile P	2.108	0.126	25.925	**<0.001**	0.383	0.883
	$NaHCO_3 P_o$	0.429	0.734	16.463	**<0.001**	0.188	0.977
	NaOH-I P_i	1.944	0.149	13.138	**<0.001**	0.507	0.797
	NaOH-I P_o	1.979	0.144	1.192	0.321	0.677	0.669
	HCl P_i	0.091	0.965	0.989	0.387	0.256	0.952
	NaOH-II P_i	0.223	0.880	11.704	**<0.001**	0.446	0.840
	NaOH-II P_o	0.485	0.696	11.865	**<0.001**	0.581	0.742
	ΣP_i	2.753	0.165	16.028	**<0.001**	0.409	0.865
	ΣP_o	0.845	0.483	6.718	**0.005**	0.370	0.891
7.5-15	Labile P	1.308	0.295	9.290	**0.001**	0.156	0.986
	$NaHCO_3 P_o$	0.469	0.706	16.292	**<0.001**	0.128	0.992
	NaOH-I P_i	1.947	0.149	25.745	**<0.001**	0.451	0.837
	NaOH-I P_o	0.994	0.412	1.066	0.360	0.612	0.718
	HCl P_i	0.253	0.859	0.254	0.778	0.135	0.990
	NaOH-II P_i	0.335	0.800	12.491	**<0.001**	0.844	0.549
	NaOH-II P_o	1.035	0.395	12.066	**<0.001**	1.046	0.421
	ΣP_i	2.100	0.127	20.076	**<0.001**	0.381	0.884
	ΣP_o	0.638	0.598	2.740	0.085	0.538	0.774
15-22.5	Labile P	0.376	0.771	7.717	**0.003**	0.211	0.970
	$NaHCO_3 P_o$	0.174	0.913	9.074	**0.001**	0.315	0.923
	NaOH-I P_i	1.751	0.183	22.226	**<0.001**	0.746	0.618
	NaOH-I P_o	1.286	0.302	2.967	0.071	0.251	0.954
	HCl P_i	0.009	0.999	0.931	0.408	0.080	0.998
	NaOH-II P_i	0.170	0.915	11.833	**<0.001**	0.623	0.710
	NaOH-II P_o	0.144	0.933	9.103	**<0.001**	1.358	0.271
	ΣP_i	0.978	0.419	11.756	**<0.001**	0.392	0.877
	ΣP_o	0.640	0.597	3.873	**0.035**	0.328	0.915
22.5-30	Labile P	0.526	0.668	13.827	**<0.001**	0.235	0.961
	$NaHCO_3 P_o$	0.484	0.697	5.400	**0.012**	0.678	0.669
	NaOH-I P_i	1.083	0.375	12.318	**<0.001**	0.603	0.725
	NaOH-I P_o	4.215	**0.016**	4.451	**0.023**	0.874	0.528
	HCl P_i	0.058	0.981	1.324	0.285	0.216	0.968
	NaOH-II P_i	0.893	0.459	5.842	**0.009**	0.181	0.979
	NaOH-II P_o	0.120	0.947	5.679	**0.010**	0.943	0.483
	ΣP_i	0.497	0.688	4.415	**0.023**	0.284	0.939
	ΣP_o	2.853	0.158	7.360	**0.003**	0.662	0.681

Accordingly, data for all soil fractions were averaged across all four understoreys and are shown for the 0-7.5 cm, 7.5-15 cm, 15-22.5 cm and 22.5-30 cm soil depths in Figures 6.2, 6.3, 6.4 and 6.5, respectively.

In general, the changes observed in each fraction and total extractable pools over the different sampling dates were similar for all depths. HCl P_i did not show significant differences between years at any depth. Labile P and NaOH-II P_i decreased significantly between 1991 and 2000 and then didn't change between 2000 and 2010, while NaOH-I P_i decreased significantly between 1991 and 2000 for all depths, and then increased significantly between 2000 and 2010 for 0-7.5 cm and 7.5-15 cm soil depths, although NaOH-I P_i determined in 2010 in the 7.5-15 cm layer remained significantly lower than 1991 (Figures 6.2 and 6.3).

For all soil depths, $NaHCO_3$ P_o increased significantly between 1991 and 2000 and did not change between 2000 and 2010. Total extractable P_o showed a similar pattern, though the difference between 1991 and 2000 in the two middle layers was not significant. Total extractable P_o was, however, significantly higher in all soil depths in 2010 than 1991. NaOH-II P_o increased significantly between 1991 and 2000, and then decreased significantly in 2010 back to levels similar to 1991, while NaOH-I P_o did not show any significant differences between years, except for 15-22.5 and 22.5-30 cm soils where concentrations determined in 2010 where higher than 2000 and 1991, respectively (Figures 6.4 and 6.5).

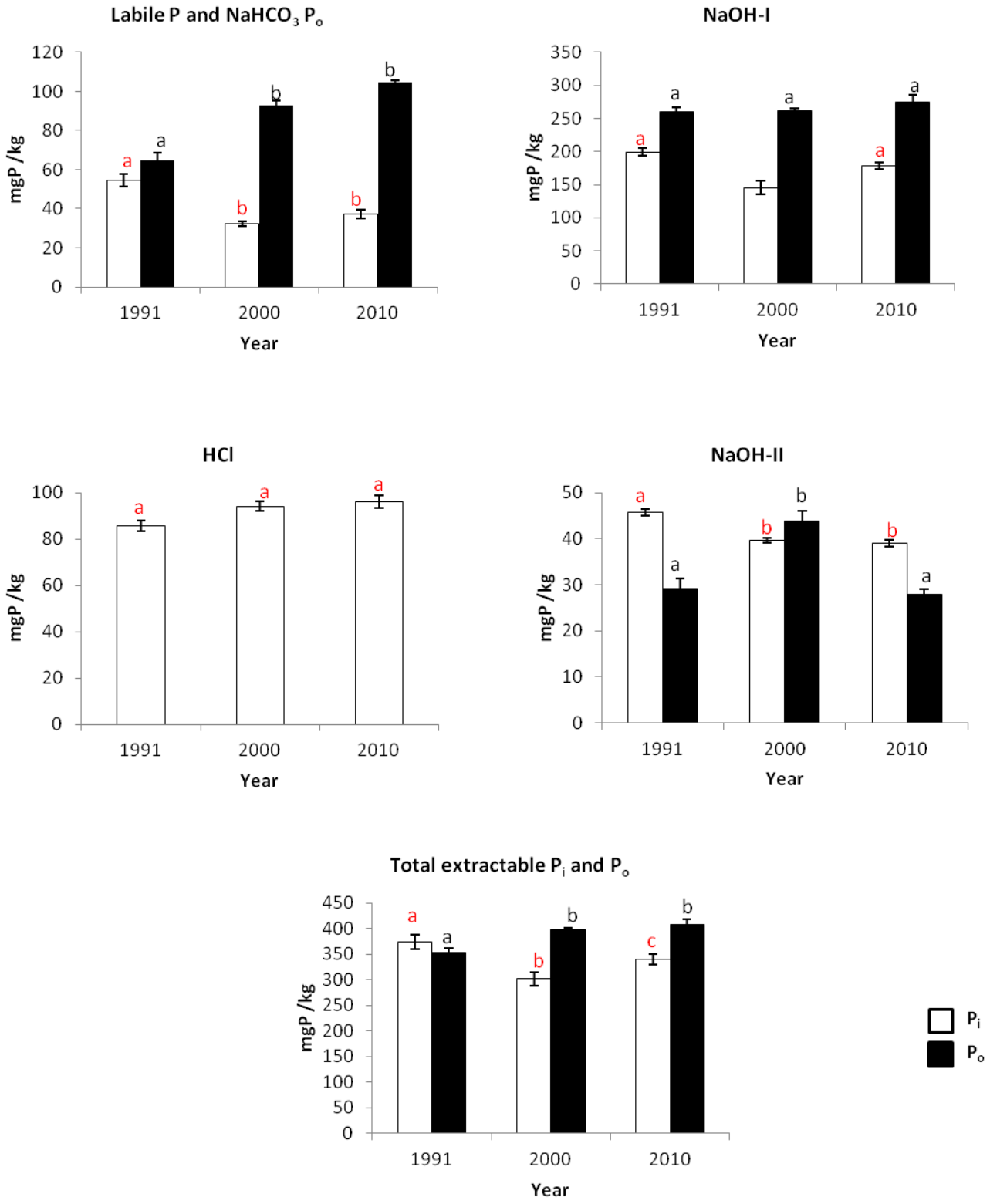

Figure 6.2 Mean P concentrations (mg P/kg) of different fractions determined in 1991, 2000 and 2010 for the 0-7.5 cm soil depth averaged over the three understorey treatments (ryegrass-clover, cocksfoot-clover and lucerne). The error bars represent standard errors of means. Different letters indicate that means of P_i or P_o fractions were significantly different between years ($p < 0.05$).

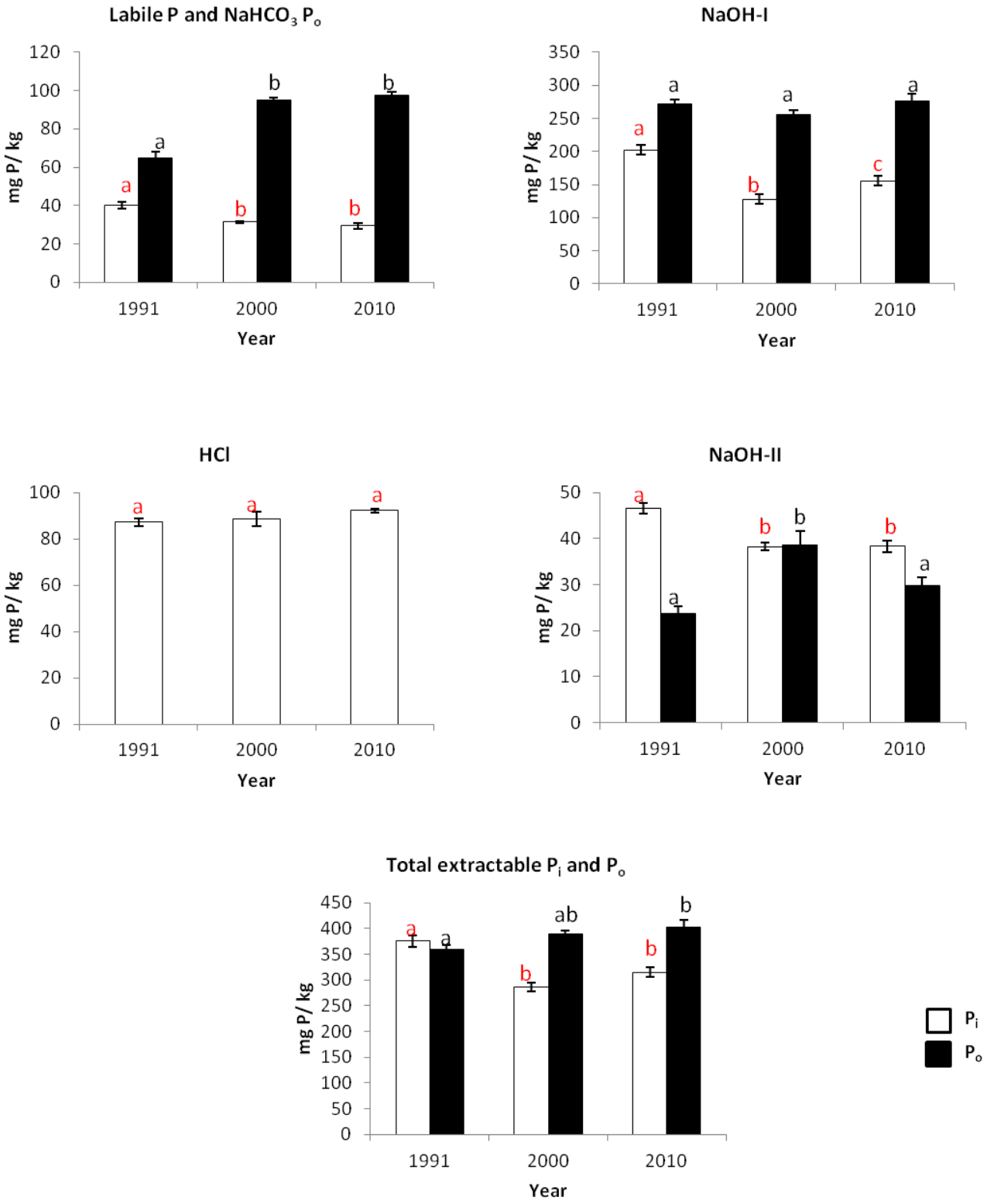

Figure 6.3 Mean P concentrations (mg P/kg) of different fractions determined in 1991, 2000 and 2010 forthe 7.5-15 cm soil depth averaged over the three understorey treatments (ryegrass-clover, cocksfoot-clover and lucerne). The error bars represent standard errors of means. Different letters indicate that means of P_i or P_o fractions were significantly different between years ($p < 0.05$).

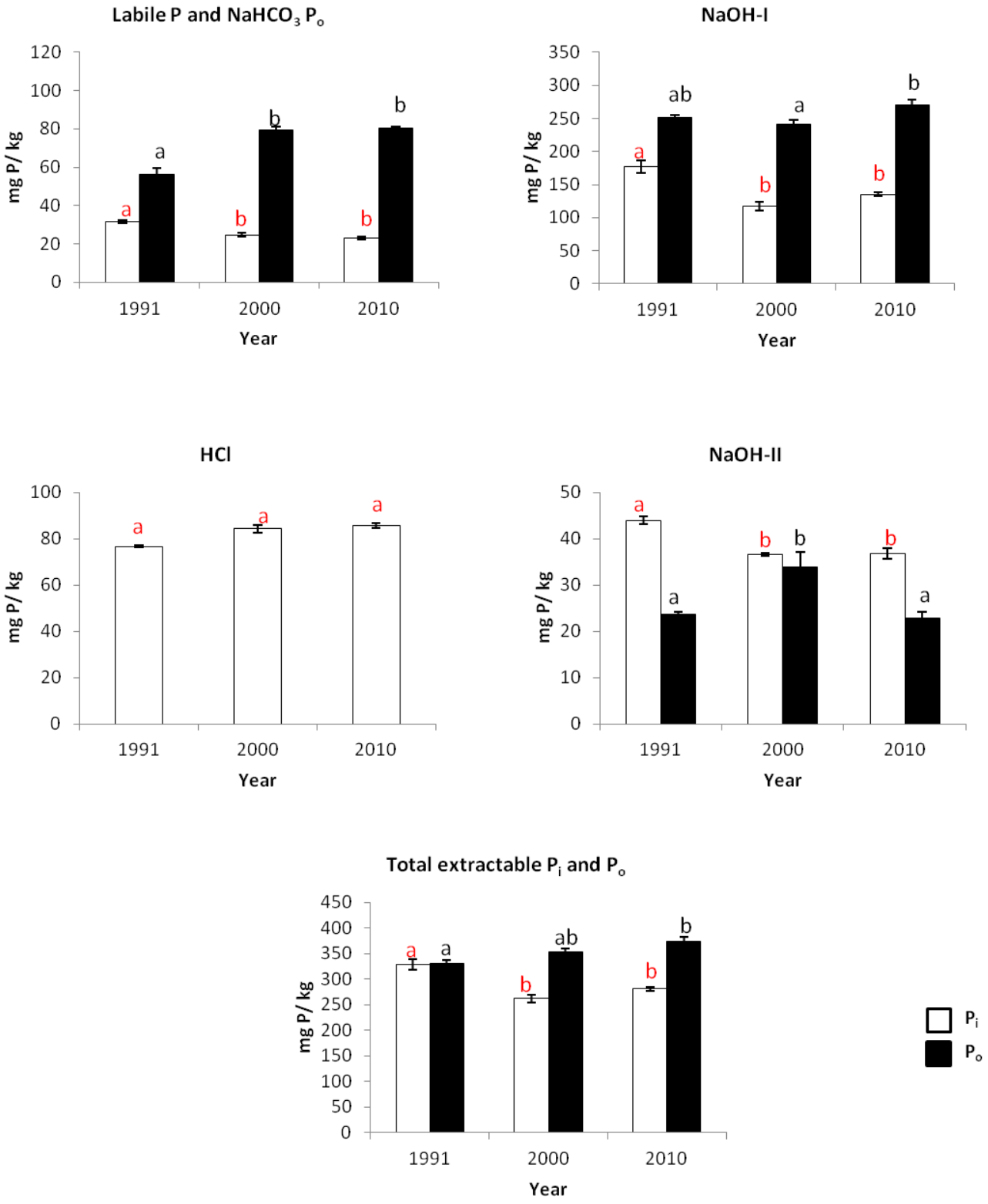

Figure 6.4 Mean P concentrations (mg P/kg) of different fractions determined in 1991, 2000 and 2010 forthe 15-22.5 cm soil depth averaged over the three understorey treatments (ryegrass-clover, cocksfoot-clover and lucerne). The error bars represent standard errors of means. Different letters indicate that means of P_i or P_o fractions were significantly different between years ($p < 0.05$).

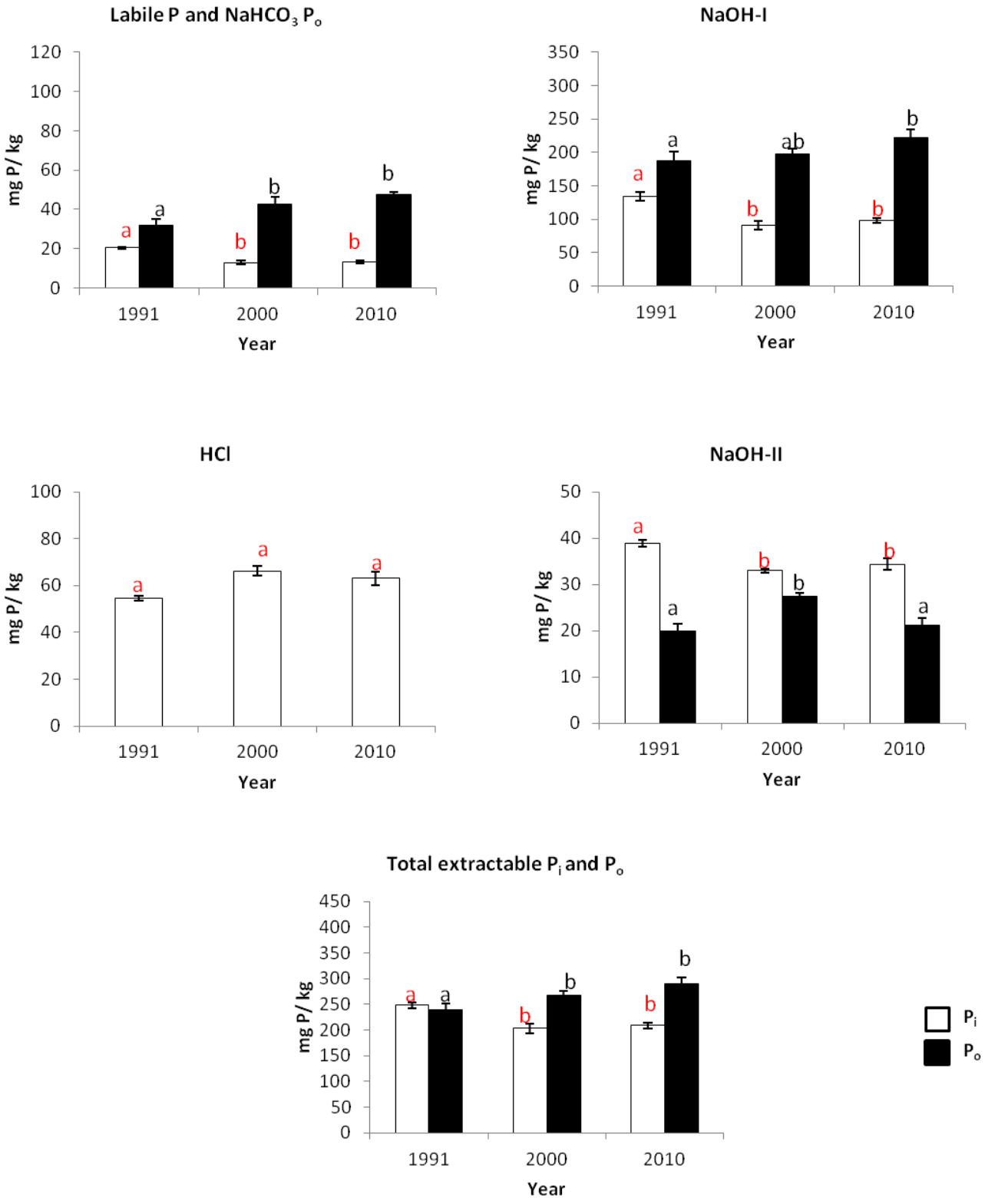

Figure 6.5 Mean P concentrations (mg P/kg) of different fractions determined in 1991, 2000 and 2010 forthe 22.5-30 cm soil depth averaged over the three understorey treatments (ryegrass-clover, cocksfoot-clover and lucerne). The error bars represent standard errors of means. Different letters indicate that means of P_i or P_o fractions were significantly different between years ($p < 0.05$).

6.3.2 Enzyme Labile Soil Organic P

Figure 6.6 shows the data for enzyme labile $NaHCO_3$-extractable organic P determined for the 0-7.5 cm soil under lucerne in 1991, 2000 and 2010. Inorganic P release was consistently higher for alkaline phosphatase (20-30 mg P/kg) compared with phytase (2-10 mg P/kg). Organic P mineralised by alkaline phosphatase increased significantly between 1991 and 2000 and then decreased in 2010 to levels that were not significantly different from those in 1991. On the other hand, organic P released by phytase increased significantly and continuously from 1991 to 2010.

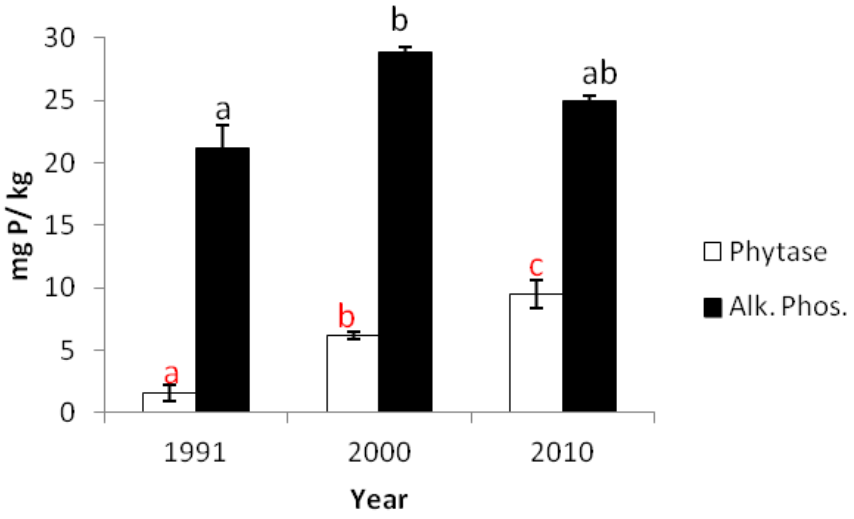

Figure 6.6 Mean concentration of inorganic P (mg P/kg) released by phytase and alkaline phosphatase in 1991, 2000 and 2010 under lucerne for the 0-7.5 cm soil depth. The error bars represent standard errors. Different letters indicate that means of phytase or alkaline phosphatase were significantly different between years ($p < 0.05$).

6.4 Discussion

It was hypothesised that the amounts and forms of soil P would change during the 19 years following the establishment of the trial, and that understorey species would have differing effects on soil P levels, due to different P-uptake strategies. While changes in soil P did occur, there were no significant differences due to understory treatments except that NaOH-I P_o in the 22.5-30 cm soil layer was higher under lucerne than under the other understory treatments.

This was surprising given that the 3 understory pastures vary in root morphology that allows them to compete differently for water and nutrients (Gautam et al., 2003). Gautam et al. (2002) investigated fine root length density, finding that for samples taken midway between tree rows, pasture plant roots predominated over *P. radiata*, while the difference was much less when samples were taken close to tree rows. This may, at least partially, explain the lack of significant differences between understory treatments in the present study, since samples were taken close to tree rows.

During the first 9 years of the trial (1991-2000) the understorey pastures established and grew very well as did the trees (Figure 6.7). During this period, intensive thinning and pruning of trees was carried out and the resulting debris was removed in order to maintain suitable conditions for pasture growth. Bandara et al. (1999) found that pruning of radiata pine at the Lincoln site improved water use efficiency of the trees which in turn helped to maintain the pasture understory.

Figure 6.7 View of the understorey in Lincoln University Silvopastoral Field Trial in 2000.

As previously mention, the field trial site was abandoned in 2000, when fences were removed, grazing management ceased, together with any further tree pruning or thinning. Continued tree growth, consequent gradual canopy closure and increased demand for soil water by the tree crop would have steadily reduced pasture growth,

together with deposition of needle litter. By 2010 the pasture understories had disappeared and the site resembled a conventional plantation forest (Figure 6.8).

Figure 6.8 View of the understorey in Lincoln University Silvopastoral Field Trial in 2010.

As Hawke (1991) explained, the decline in pasture production with increasing forest age is a result of the direct competition for light, soil nutrients and water. Understory pastures are also sensitive to silvicultural practices due to debris from thinning and pruning which reduces the area available for grazing. The rate of breakdown is dependent on the size of the debris and the stock species used for grazing, although this was not the case for the present study.

Knowles *et al*. (1999) developed a canopy closure model to predict understorey relationships in *P. radiata* silvopastoral systems. They reported that their model indicated 100 % pasture presence when trees did not produce shade at the early stages of the stand growth, but at approximately 70% of canopy closure, predicted zero pasture production. This was attributed to various factors including the quantity and quality of the light, more acidic soil conditions under *P. radiata* and greater competition for nutrients between trees and understorey vegetation.

The discussion below will focus on changes in soil P that were determined between 1991-2000 and 2000-2010, that is 9 and 19 years after trial establishment.

6.4.1 1991 to 2000

The significant decreases in soil inorganic P (labile, NaOH-I and NaOH-II P fractions) together with concomitant increases in soil organic P (predominantly $NaHCO_3$ and NaOH-II fractions) that occurred between 1991 and 2000 clearly demonstrated that establishment and initial development of the silvopastoral system had resulted in net biological immobilization of P in the soil. As noted previously soil sampling adjacent to the tree rows was designed to provide a measure of the combined impact of trees and pasture establishment and growth on soil P. The increase in soil organic P that occurred adjacent to the tree rows over the first 10 years after tree establishment was surprising, based on findings obtained from numerous paired-site comparison afforestation studies, mainly performed in New Zealand (Amatya et al., 2002; Scott and Condron, 2003). For example, Scott and Condron (2004), conducted a glasshouse experiment to determine the short term effects of *P. radiata*, lucerne and ryegrass on plant P uptake and the specific mineralisation rate, and they found that the combination of the species tended to deplete more inorganic P than under pastures alone, and although P uptake was greater under *P. radiata*, the trees tended to deplete inorganic P to a lesser extent than the forages.

Results from a wide range of sites have demonstrated that concentrations and amounts of soil organic P were consistently significantly lower under recently established forest compared with adjacent grazed and ungrazed pasture (Chen et al., 2008). Furthermore, results obtained from the Orton Bradley Park tree species trial confirmed that significant decreases in soil organic P occurred within 5 years of tree planting (see Chapter 3). The fact that soil organic P increased during the first 10 years of the Lincoln silvopastoral field trial can be attributed to increases in soil organic matter and organic P associated with establishment of semi-permanent grazed pasture in a relatively high fertility soil which, prior to 1990, had been under an intensive rotational crop-pasture system for many years. Numerous studies carried out in New Zealand and elsewhere have shown that soil organic matter and organic P increase significantly as a consequence of establishment of improved grazed pasture with fertilizer inputs (Ann et al., 1999; Condron and Goh, 1989a; Condron and Tiessen, 2005). This is confirmed by the small but significant increase in

soil organic C that occurred at the Lincoln silvopastoral site between 1991 and 2000 (Table 6.3). The increased level of organic P in the soil that occurred as a consequence of pasture establishment was also reflected in the significant increase in enzyme-labile monoester organic P between 1991 and 2000.

The fact that labile, NaOH-I P_i, NaOH-II P_i and total extractable P_i decreased significantly between 1991 and 2000 coupled with the increase of $NaHCO_3$ P_o, NaOH-II P_o and total extractable P_o, indicated that net immobilisation of soil inorganic P occurred during the first 9 years of establishment. As suggested, this can be attributed to the highly fertile soil conditions that existed prior to the trial establishment, induced by the application of fertilizers which could have increased inorganic P levels and decreased the mineralization rate leading to an accumulation of organic matter from understorey plants and *P. radiata* inputs. Another contributing factor could be the effect of grazing, which as explained in Chapter 2, enhances the turnover and accumulation of organic P via excreta.

Table 6.3 Percentage of total C, total N and C:N ratio in soil (0-7.5 cm) determined in 1991, 2000 and 2010 averaged over the three understorey treatments (ryegrass-clover, cocksfoot-clover and lucerne).

Year	%N	%C	C:N
1991	0.21	2.98	14.19
2000	0.22	3.45	15.68
2010	0.22	3.21	14.59

6.4.2 2000 to 2010

The absence of any significant changes in $NaHCO_3$ P_o, NaOH-I P_o and total extractable P_o between 2000 and 2010 compared with the 1991-2000 period, and the small but significant increase in extractable soil inorganic P (mainly NaOH-I fraction in the upper two layers), may be attributed to a combination of factors related to changes in the management of the trial site post 2000. As explained above, continued tree growth and resulting canopy closure following abandonment of the trial in 2000 would have led to declining pasture production and eventually to its demise. Peri et

al. (2002) quantified the effect of the understorey species on *P. radiata* growth and quality at the same study site. They reported that trees growing with no-understorey showed up to 34% higher volume and were 15% higher than trees growing with a pasture understory, which they attributed to competition for water. This suggests that when the grasses disappeared after the canopy closure, the pine growth was enhanced and this lead to the accumulation of organic matter, as shown in Table 6.3.

The significant increase in soil inorganic P was confined to the top 15 cm suggesting that it may have originated from the decomposition of pasture biomass as a consequence of increased competition from trees. Consistent with findings from the Orton Bradley Park trial on the impacts of cessation of grazing on soil P, it would be expected that the decline and demise of the pasture at Lincoln would also have resulted in a decrease in soil organic P. The small but significant decreases in NaOH-II P_o that occurred in all soil depths between 2000 and 2010 provides some evidence of this. Anecdotal evidence suggest that remnant pasture understories were still present at the site up to 2008, and so significant decreases in soil organic P could be predicted after 2010, as decomposition of the residual pasture understory vegetation proceeds. However, it is also possible that continued inputs of organic matter and P from the trees will also affect the amounts and forms of future organic P in the soil, especially as the soil pH declines further with forest development (Table 6.4).

Table 6.4 Soil pH (0-7.5 cm) determined under the understory treatments in 1991, 2000 and 2010 (standard errors are shown in parenthesis).

	Ryegrass-clover	Cocksfoot-clover	Lucerne
1991	6.0	5.8	5.9
	(0.09)	(0.08)	(0.14)
2000	5.7	5.6	5.8
	(0.07)	(0.11)	(0.13)
2010	5.6	5.4	5.4
	(0.08)	(0.03)	(0.11)

The above suggest that after abandonment, the system started to exhibit characteristics more similar to those found in a forestry system than a grazed pasture.

6.5 Conclusion

The results confirmed that significant net immobilisation of soil organic P occurred following the simultaneous establishment of widely spaced *P. radiata* and grazed pasture on a low organic matter/high P soil, although the trends also indicated that the immobilisation process stabilized after the abandonment of the trial. Significant differences in the P fractions between the different understories did not occur during the course of the trial. This suggests that the immobilisation observed was associated with establishment of semi-permanent grazed pasture in a soil of relatively high fertility, previously under an intensive rotational crop-pasture system for many years prior to trial establishment, and an accumulation of organic matter from understorey pasture together with inputs of excreta from grazing animals. No further increase of organic P occurred between 9-19 years, and it is possible that future changes in soil P will be determined by the effects of continued tree growth and P uptake following the demise of the pasture understory.

CHAPTER 7 BIOAVAILABILITY OF PHOSPHORUS IN SOILS TAKEN FROM A NATIVE FOREST CHRONOSEQUENCE

7.1 Introduction

Different mycorrhizal associations (e.g. ectomycorrhizae vs arbuscular mycorrhizae) are likely to influence plant P acquisition from soil (Chen et al., 2008), which in turn will be affected by the chemical nature and relative solubility of P in soil (e.g. inorganic vs organic). Data obtained from soil chronosequences have clearly demonstrated that the quantity and nature of soil P change dramatically during long-term ecosystem development gradients (Peltzer et al., 2010; Richardson et al., 2004). Soil chronosequences provide an ideal template to investigate the relative bioavailability of soil P to different plant species. The specific objective of this study was to investigate and compare P uptake and acquisition by contrasting commercial plantation forest tree species, *P. radiata* (ectomycorrhizal) and *C. macrocarpa* (arbuscular mycorrhizal), from a series of soils taken from the 6,500 year-old coastal sand dune chronosequence developed under native New Zealand forest.

7.2 Materials and methods

7.2.1 Haast chronosequence

The Haast chronosequence is a foredune ridge system located in south Westland, New Zealand at 43 º 43’ S; 169 º 4’ E (Turner et al., 2012) (Figure 7.1). The chronosequence was most likely the result of episodic earthquakes along the Alpine Fault which caused landslides and floodplain aggradation releasing sediments in the Haast river that are eventually deposited in a linear dune at each side of the mouth of the river (Wells and Goff, 2006). The dunes are younger towards the ocean, progressively increasing in age while moving inland, and extended approximately 20 km parallel to the coast. The dune system consists of a series of around 20-100 m wide sand dunes, separated by poorly drained swales (Eger *et al*., 2011; Wells and Goff, 2006). Wells and Goff (2006) dated some dunes using tree rings and historical records and reported that the youngest dune was formed circa 1826, while the register for the oldest dune goes 6500 years before present (Figure 7.6). According

to Eger *et al*. (2011), the particle size distribution of the parent sand is similar across the chronosequence and it mainly consists of glacially produced particles of quartzo-feldspathic gneiss of the Greenland group. Further information about the soil chemical and physical properties of the dunes is shown in Table 7.1. Mean annual temperature range is 11.3°C and a mean annual rainfall of 3455 mm (Eger *et al*., 2011). Vegetation consists on a mixed podocarp -*Nothofagus*- broad leaved rain forest, with *Dacrydium cupressinum* (rimu), *Prumnopitys ferruginea* (miro) and *Podocarpus hallii* (totara) being particularly abundant (Coomes and Bellingham, 2011). The woody angiosperms *Weinmannia racemosa* (kamahi), *Coprosma* spp., *Nothofagus menziessi* (silver beech) and tree ferns such as *Dicksonia squarrosa* and *Cyathea smithii* are also abundant, while vegetation in swales is mainly composed by herbaceous wetlands (Dickinson and Mark, 1994) (Figure 7.2). The youngest dune is the exception because it was cleared, converted to pasture and eventually abandoned. Nevertheless some low stature forest remnants still persist on the dune crests (Wells and Goff, 2006).

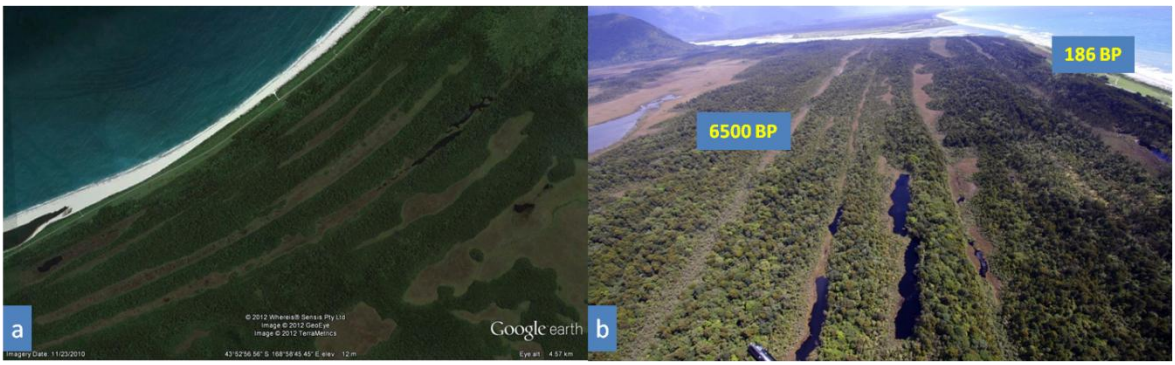

Figure 7.1 a) Satellite view of field research site at Haast Chronosequence (source: Google Earth) and b) aerial view of the Haast Chronosequence looking south, with the youngest dune at the right (181 years before present) and the oldest at the left (6500 years before present).

Table 7.1 Description of soils from profile pits of each of five dune stages along the Haast dune chronosequence (Turner *et al.*, 2012).

Dune	Dune age (B.P.)	Soil taxonomy	Dune height m	Topsoil texture	Eluvial horizon	Iron pan	Cation exchange capacity $cmol_c\ kg^{-1}$
1	181	Typic Udipsamment	3	Sand	No	No	-
2	392	Typic Udipsamment	3-4	Loamy sand	No	No	1.3
3	1826	Spodic Udipsamment	5	Loamy sand	Yes	No	2.7
4	4422	Typic Placorthod	4	Loamy sand	Yes	Yes	2.1
5	6,500	Typic Placorthod	15	Loamy sand	Yes	Yes	0.7

Figure 7.2 View of the vegetation on different dunes of Haast chronosequence. a) dune 1 (± 181 years old), b) dune 2 (± 392 years old), c) dune 4 (± 4,422 years old) and d) dune 5 (± 6,500).

7.2.2 Soil Sampling, Pot Experiment and Analyses

Approximately 500 grams of mineral soil from the top 20 cm was collected from five points randomly located on five selected dunes of different ages ranging from 181 to 6,500 years old (i.e. Dunes 1-5 - see Table 7.1). Soil samples from each dune were bulked, air-dried at 30 °C, sieved (< 2 mm) and stored in plastic bags. Seeds of *P. radiata* and *C. macrocarpa* were germinated and when the seedlings were approximately 2 cm, they were directly planted into pots containing 100 grams of soil from each dune (six seedlings per pot). Pots containing *P. radiata* were inoculated with ectomycorrhizal fruiting bodies, while those containing *C. macrocarpa* were inoculated with endomycorrhizal spores (both obtained from the field). There were five replicates for each treatment, and the trial ran for six months in a glasshouse (Figure 7.3).

Figure 7.3 Pot experiment design: *P. radiata* seedlings are shown at the left side and *C. macrocarpa* seedlings at the right side. Within each group, pots containing soil from dunes 1 to 5 are shown at the front row from left to the right.

At the end of the experiment, mineral soil attached to the roots was collected by shaking the seedlings, and plant shoots and roots were removed, divided and oven dried at 60º C for 72 hours. Plant P was measured on a dry basis with an ICP –OES (Varian 720 Australia Ltd).

Fresh mineral soil from the pots of each batch of dunes was bulked together and used to determine enzyme-labile soil organic P (as described in Chapter 4). Subsamples of original field soils and samples taken from pots at the experiment harvest were finely ground prior to detailed soil P fractionation analysis (Section 4.2.1.1).

7.2.3 Statistical Analysis

Statistical analyses were performed using R program (2012, The R Foundation for Statistical Computing). One way analyses of variance were performed to test the significance of treatment (pre-experiment, *P. radiata* and *C. macrocarpa*). Where F ratios were significant ($P< 0.05$) treatment means were compared by the Least Significant Differences (*lsd*) in a general ANOVA.

7.3 Results

The quantities and distribution of soil P fractions for the 5 Haast dunes are presented in Figure 7.4. These data clearly show that concentrations of total extractable P_i decreased dramatically between dunes 1 (279 mg P/kg) and 2(45 mg P/kg), while total extractable P_o increased from 44 mg P/kg in dune 1 to 153 mg P/kg in dune 2. Note that there was an overall decrease in total extractable P (i.e. $P_i + P_o$) of 323 to 198 mg P/kg between dunes 1 and 2. The decline in inorganic P that occurred between dunes 1 and 2 were mainly in the labile and NaOH-I fractions, while concomitant increases in organic P occurred in the NaOH-I and NaOH-II fractions. Concentrations of total extractable P_i and P_o decreased steadily between dunes 2 and 5 from 45 to 17 mg P/kg for inorganic P and from 153 to 91 mg P/kg for organic P.

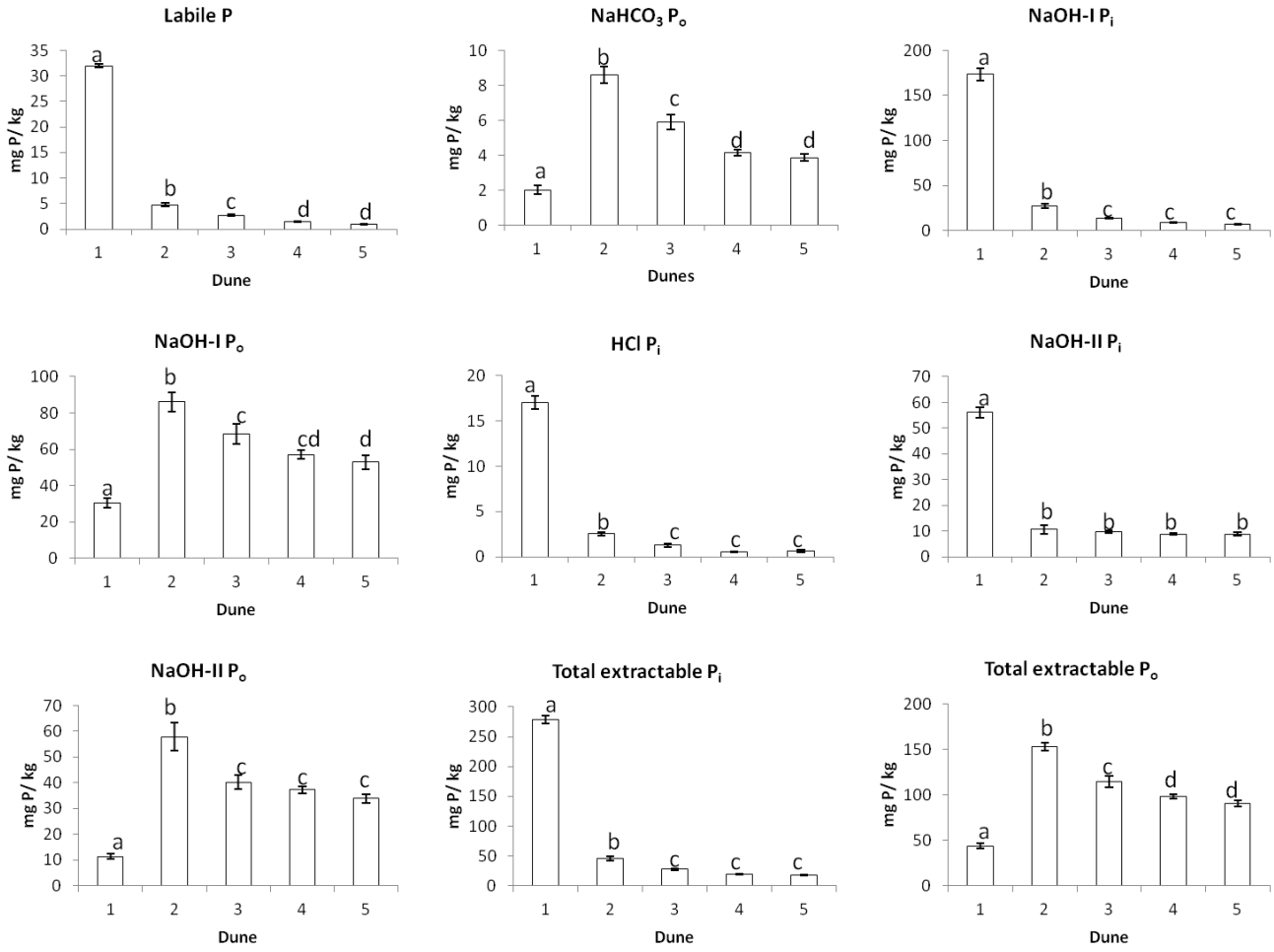

Figure 7.4 Mean data for soil P fractions and total extractable inorganic and organic P (mg P/kg) determined in soils before the experiment for each dune (1=181 years; 2=392 years; 3=1826 years; 4=4422 years; 5=6500 years). The error bars represent standard errors of means. Different letters indicate that means were significantly different between dunes ($p < 0.05$).

Table 7.2 shows the root and shoot dry matter yields, plant P concentration and uptake data for *P. radiata* and *C. macrocarpa* for the 5 Haast soils. These results revealed that yield and P uptake were higher for *P. radiata* than *C. macrocarpa* across all soils, except for P uptake for the dune 5 soil. Yields for both plant species were lower for dune 1 soil compared with dunes 2-5, and although P uptake for *P. radiata* was similar for all 5 soils, P uptake for *C. macrocarpa* was lower for soils from dunes 1 and 4 compared with the rest.

Table 7.2 Mean data for a) Root, shoot and total biomass (dry weight g pot^{-1}), and shoot root ratios (S:R), of *P. radiata* (P.R.) and *C. macrocarpa* (C.M.) and b) P uptake by *P. radiata* and *C. macrocarpa* determined after 6 months growth for all the Haast chronosequence soils.

a)

Dune	Root		Shoot		Total		S:R ratio	
	P.R.	C.M.	P.R.	C.M.	P.R.	C.M.	P.R.	C.M.
1	1.5	0.9	3.8	2.0	5.3	2.9	2.6	2.2
2	2.0	2.2	5.7	5.0	7.7	7.2	2.8	2.3
3	2.2	2.0	5.4	4.2	7.6	6.2	2.4	2.1
4	2.0	1.3	5.5	3.2	7.5	4.5	2.8	2.5
5	2.0	2.0	5.9	4.8	7.9	6.8	3.0	2.4
Mean	1.9	1.7	5.3	3.8	7.2	5.5	2.7	2.3
S.E.	0.1	0.2	0.2	0.4	0.5	0.8	0.1	0.1

b)

Dune	P concentration (mg P/kg^{-1})				P uptake (mg pot^{-1})					
	Root		Shoot		Root		Shoot		Total	
	P.R.	C.M.	P.R.	C.M.	P.R.	C.M.	P.R.	C.M.	P.R.	C.M.
1	629.5	620.5	617.4	573.7	0.93	0.56	2.36	1.16	3.3	1.7
2	441.7	491.7	433.2	454.6	0.90	1.06	2.47	2.27	3.4	3.3
3	407.3	425.4	399.5	393.3	0.90	0.84	2.16	1.65	3.1	2.5
4	419.6	372.5	411.5	344.4	0.82	0.48	2.26	1.10	3.1	1.6
5	339.2	435.1	332.7	402.3	0.66	0.87	1.96	1.93	2.6	2.8
Mean	447.5	469.0	438.9	433.6	0.84	0.76	2.24	1.62	3.1	2.4
S.E.	48.61	48.8	43.2	45.6	0.08	0.08	0.22	0.72	1.0	0.5

Changes in concentrations of various soil P fractions that occurred as a result of growing *P. radiata* and *C. macrocarpa* for 6 months in the 5 Haast soils are shown in Figure 7.5. For the dune 1 soil, labile P was significantly lower after growth of *P. radiata* compared with *C. macrocarpa* and the original soil, although no similar differences were apparent for the other soils. Concentrations of $NaHCO_3$ P_o, NaOH-I P_o and NaOH-II P_o in soils from dunes 2-5 were significantly lower after plant growth compared with corresponding original soils, although concentrations determined for dunes 2, 3 and 5 were similar for *P. radiata* and *C. macrocarpa* For the dune 4 soil, concentrations of $NaHCO_3$, NaOH-I and NaOH-II P_o were all significantly lower for *P.*

radiata compared with *C. macrocarpa*. These trends were reflected in data for total extractable inorganic and organic P.

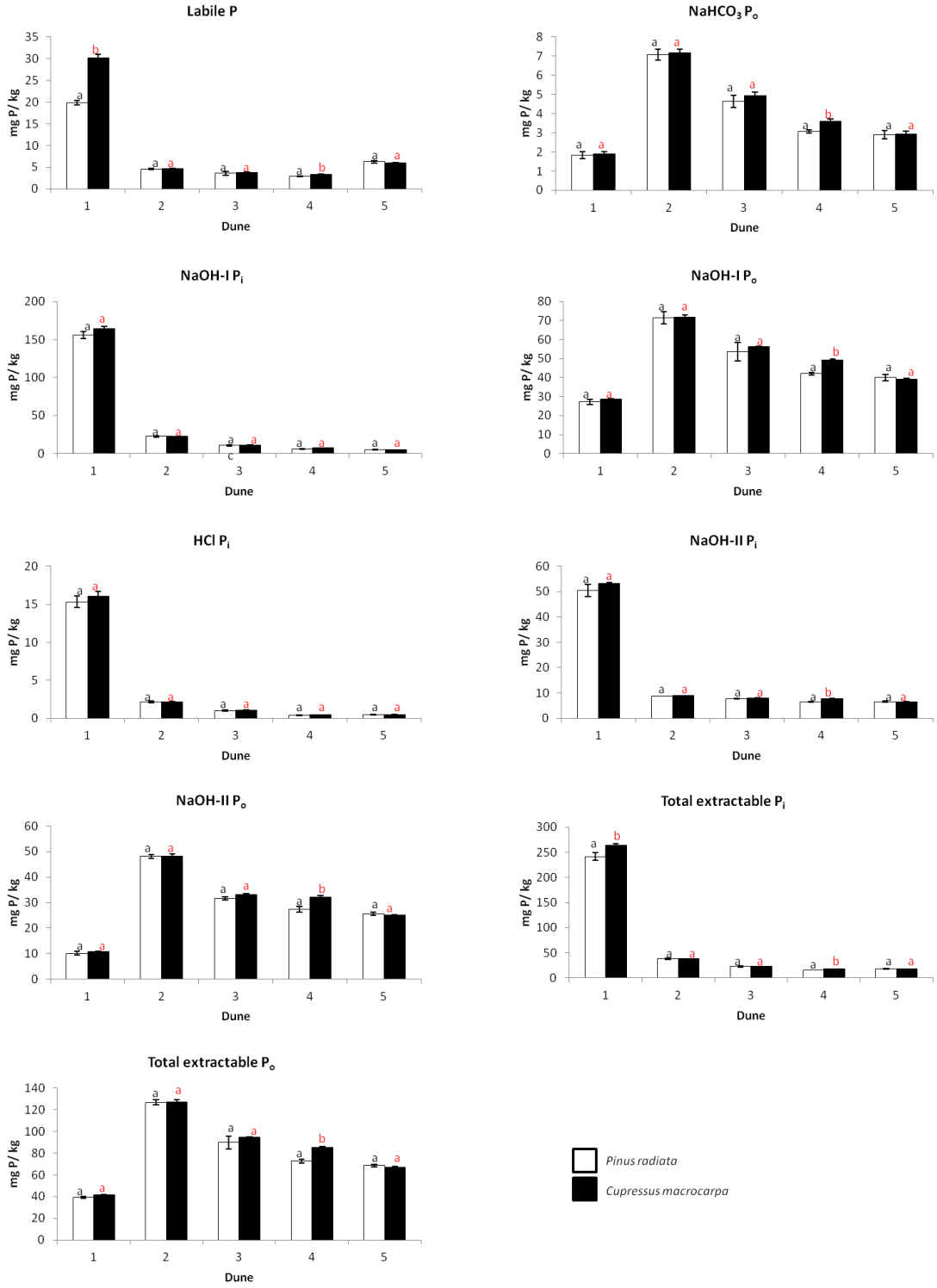

Figure 7.5 Mean data for soil P fractions and total extractable inorganic and organic P (mg P/kg)determined in soils under *P. radiata* and *C. macrocarpa* from each dune (1=181 years; 2=392 years; 3=1826 years; 4=4422 years; 5=6500 years). The error bars represent standard errors of means. Different letters indicate that means were significantly different between treatments ($p < 0.05$).

Data on enzyme labile $NaHCO_3$ extractable organic P for each soil after six months growth under *P. radiata* and *C. macrocarpa* are shown in Figure 7.6. Consistent with findings from other studies reported in this thesis, quantities of inorganic P released by alkaline phosphatase were consistently higher (10-22 mg P/kg) than phytase (4-11 mg P/kg). Alkaline phosphatase inorganic P release was higher from dunes 2-4 compared with dune 1 and 5, while phytase inorganic P was higher for dunes 2 and 3 compared with 1, 4 and 5. Significant differences were observed between *P. radiata* and *C. macrocarpa* for both enzymes across all soils, except phytase inorganic P released for dune 2. Alkaline phosphatase labile organic P was greater under *C. macrocarpa* compared with *P. radiata* for all dunes except 4, while phytase labile organic P was greater under *P. radiata* compared with *C. macrocarpa* for dunes 3, 4 and 5.

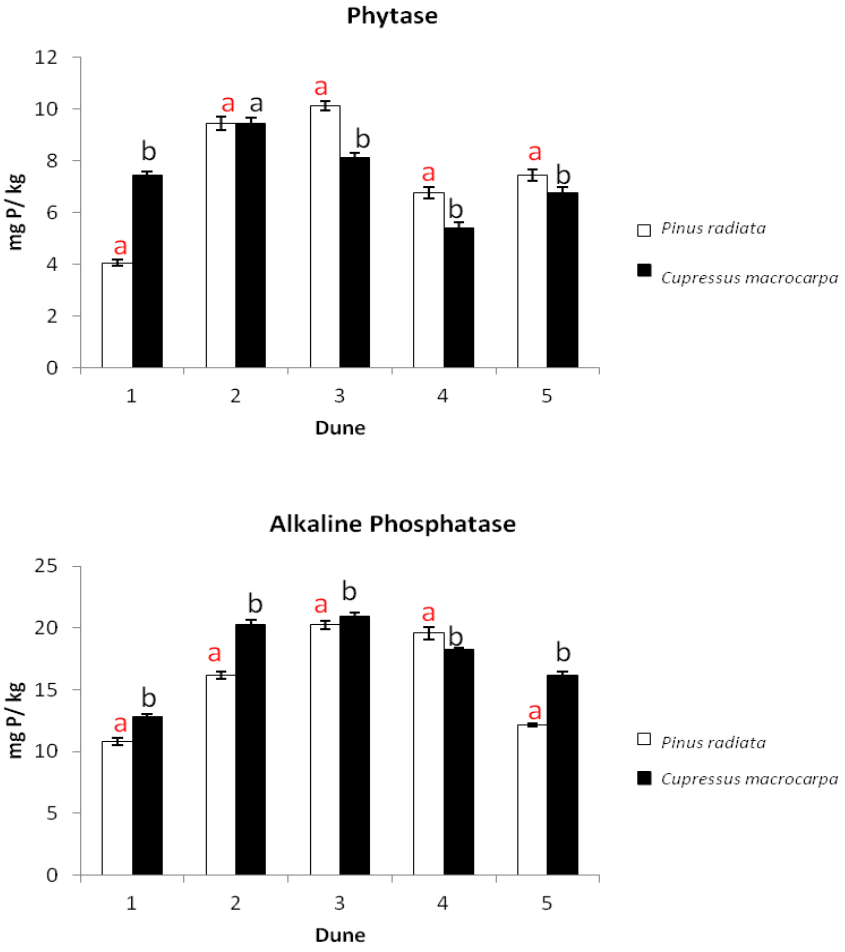

Figure 7.6 Mean concentration (mg P/kg) of inorganic P released by phytase and alkaline phosphatase under *P. radiata* and *C. macrocarpa* for each dune (1=181 years; 2=392 years; 3=1826 years; 4=4422 years; 5=6500 years). The error bars represent standard errors of means. Different letters indicate that means were significantly different between species ($p < 0.05$).

7.4 Discussion

7.4.1 Changes in Soil P Fractions along the Chronosequence

It was hypothesised that the amounts and forms of soil P would change from the youngest to the oldest dune. This study showed that inorganic P decreased from the youngest to the oldest dune, while total extractable organic P increased greatly between dunes 1 and 2 and then decreased with time. This supports the general trend that has been reported by other studies in different chronosequences around the world, which indicates that the higher availability of inorganic P in young soils allows an initial enrichment of P in organic matter that eventually decreases with time due to several factors including plant uptake, organic P immobilization, leaching and erosion (Lichter, 1998; Parfitt et al., 2005; Thompson, 1981; Turner et al., 2012; Vitousek and Farrington, 1997).

The rapid decline of the inorganic P fractions and total extractable inorganic P during the early stages of the chronosequence indicates that in the young soils depletion of inorganic P occurred as a consequence of dissolution, plant uptake, microbial immobilisation and loss by leaching. On the other hand, the fact that these fractions did not change between the two oldest dunes of the chronosequence, suggests that dune 4 reached a near-terminal state of low soil fertility caused mainly by P limitation, supporting the results reported by Turner et al. (2012) for the same chronosequence, who found that total inorganic P (obtained by the ignition method) reached the lowest levels in the oldest dunes. It is, however, interesting to note that the concentration of inorganic P in the HCl fraction was low in the dune 1 soil compared to the other inorganic P fractions. The dune 1 soil was 181 years old when sampled and the inorganic P fraction results indicate that primary apatite inorganic P in the original parent material dissolved readily during the early stages of soil development and was retained in the soil as secondary mineral forms of inorganic P in the labile, NaOH-I and NaOH-II pools. This is consistent with the low pH of the dune 1 soil (4.9) (Figure 7.7). During subsequent development it appears that most of the inorganic P present in the dune 1 soil was either immobilised as organic P or lost by leaching. The steady decline in extractable organic P that occurred between

dunes 2 and 4 clearly indicates significant losses of organic P with time which was not unexpected given the free draining nature of the soil and the high rainfall.

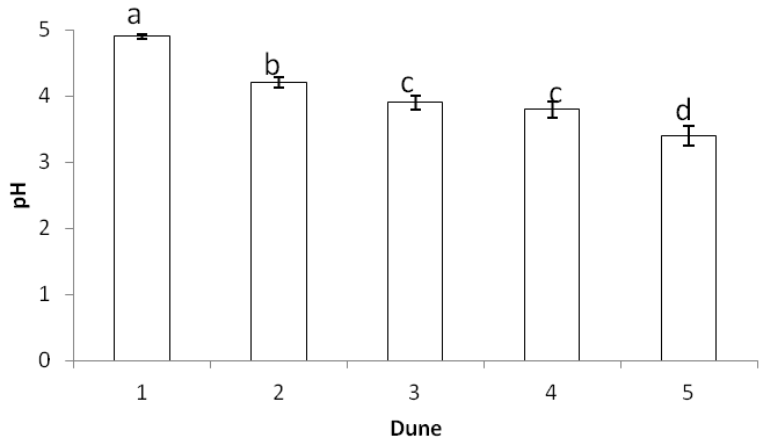

Figure 7.7 Soil pH (0-20 cm) determined before the pot experiment for each dune (1=181 years; 2=392 years; 3=1826 years; 4=4422 years; 5=6500 years). The error bars represent standard errors of means. Different letters indicate that means were significantly different between dunes ($P < 0.05$).

7.4.2 Effects of *Pinus radiata* and *Cupressus macrocarpa* Growth on Soil P

It was hypothesised that different alterations in soil P would be observed for the two exotic tree species grown, as a result of different P uptake strategies. It was not considered valid to compare the concentrations of P in the original soils with the same soils after 6 months growth of *P. radiata* and *C. macrocarpa*, since the specific objective of the study was to compare the contrasting tree species impacts on soil P.

Results from dune 1 soil indicate that *P. radiata* was able to access labile P to a greater extent than *C. macrocarpa*, which may be attributed to differences in mycorrhizal associations. This has been reported by other studies which indicate that the association between conifers and ectomycorrhizae (ECM) tends to be more efficient in P uptake than vesicular arbuscular mycorrhizae (VAM) because they can degrade cellulose in sterile conditions and phosphohydrolase production is activated even in the absence of P (Chen et al., 2004; Chen et al., 2008). Some studies have also provided evidence that ECM release significant quantities of low molecular weight organic acids which promote solubilisation of recalcitrant forms of organic P and its consequent mineralization (Chen et al., 2003; Chen et al., 2008; Cieslinski et al., 1998). However, this was not consistent with results shown here, with no

difference observed between *P. radiata* and *C. macrocarpa* for all the other P fractions in dune 1.

The fact that the concentrations of P in all fractions for dunes 2, 3 and 5 were similar under *P. radiata* and *C. macrocarpa* clearly indicates that both tree species accessed similar forms of soil P, at least over the short-term under controlled conditions. Since most of the P in these soils was present in organic forms, this suggests that access to and uptake from organic forms of P was similar for both species. This is contrary to findings from other pot trial studies that compared soil P changes associated with growth of endomycorrhizal versus ectomycorrhizal plants. For example, Chen et al. (2003) conducted a glasshouse experiment to compare changes in soil P under *Lolium perenne* (endomycorrhizal) and *P. radiata* (ectomycorrhizal) and found that *P. radiata* produced more root exudates than ryegrass, which enhanced the dissolution of iron, aluminium and calcium minerals, and in turn increased P mineralization and its subsequent uptake. Also, the apparent lack of significant difference between the two tree species could be attributed to the relatively short duration of the experiment which may have not been long enough to generate sufficient differences. This is also supported by the lack of any differences in soil pH between species after six months growth (Figure 7.8).

The increase in the concentrations of organic P in all fractions between dunes 1 and 2 and the subsequent decrease with dune age, is also reflected in the concentration of $NaHCO_3$-P_o released by phytase and alkaline phosphatase (Figure 7.6), showing an enrichment of inorganic P in the organic matter eventually decreasing with time. The fact that inorganic P released by alkaline phosphatase was generally lower in soils after 6 months growth of *P. radiata* compared with *C. macrocarpa* (except for dune 4), may be attributed to enhanced utilisation of $NaHCO_3$-extractable organic P by the former species. In other words this data tentatively suggests that *P. radiata* had accessed and depleted more labile organic P than *C. macrocarpa*. Differences between species for phytase labile P were less clear, although it should be noted that quantities of inorganic P released from the Haast soils were much lower for phytase (< 10 mgP / kg) than for alkaline phosphatase (>10 mgP/kg).

It is interesting to note that the only differences in organic P were observed for the dune 4 soil where concentrations of $NaHCO_3$, NaOH-I and NaOH-II-P_o were significantly lower for *P. radiata* compared with *C. macrocarpa*. This suggests that *P. radiata* enhanced mineralisation of organic P in this soil, which is consistent with the fact that P uptake by *P. radiata* was also markedly higher than *C. macrocarpa*. However, this is not consistent with the higher concentrations of enzyme labile P measured for *P. radiata*. The contrasting results obtained for the dune 4 soil may also indicate a marked difference in the composition and bioavailability of organic P in this soil compared with soils from dunes 2, 3 and 5.

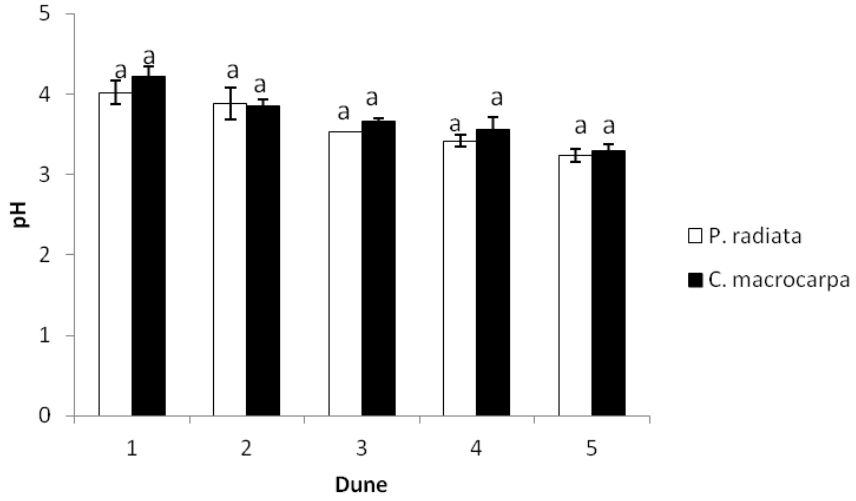

Figure 7.8 Mean data for soil pH determined after six months growth of *P. radiata* and *C. macrocarpa* for each dune (1=181 years; 2=392 years; 3=1826 years; 4=4422 years; 5=6500 years). The error bars represent standard errors of means. Different letters indicate that means were significantly different between dunes ($P < 0.05$).

7.5 Conclusions

The findings of this study confirmed that rapid biological immobilisation of inorganic P occurred during initial ecosystem development at Haast, while significant losses of inorganic and organic P occurred over 6,500 years. Results from the short-term pot experiment revealed that *P. radiata* was able to access and utilise labile inorganic P to a greater extent that *C. macrocarpa*, which might be attributed to differences in mycorrhizal associations. On the other hand, it appeared that both tree species were able to utilise organic P to a similar extent over 6 months, except for one soil (dune 4) where *P. radiata* caused greater depletion of extractable forms of organic P than *C. macrocarpa*.

CHAPTER 8 SUMMARY, CONCLUSIONS AND FUTURE RESEARCH

8.1 Summary

Herewith a summary of the major findings to emerge from the five component studies of this PhD project.

Results from the Orton Bradley Park temporal study revealed that significant decreases in soil organic P occurred during the 5 years following tree planting, which indicated net mineralisation of organic P associated with tree establishment and growth. Soil organic P decreases after 5 years were similar for *P. radiata, C. macrocarpa and E. nitens*, despite differences in mycorrhizal associations, growth rates and understorey impacts associated with each tree species. This suggested that P acquisition from soil by enhanced organic P mineralisation was similar for all three species, although this effect may also be partly attributed to reductions in organic matter P inputs and P turnover associated with cessation of grazing when trees were established. The fact that significant small increases in soil organic P occurred between 5 and 10 years after tree planting was mainly attributed to increasing above-ground and below-ground inputs of organic matter and P from trees with time which was confirmed by increases in more recalcitrant (less soluble) organic P forms over this period.

The 1-year seasonal study carried out on the same trial at Orton Bradley Park 12-13 years after establishment measured significant changes in soil P between seasons, principally accumulation of organic P during colder months and enhanced mineralisation of organic P in spring-summer. Consistent with results obtained from the longer-term temporal study, no differences were observed between species, which indicated that differences in temperature and soil moisture had a greater impact on soil P dynamics than tree species.

Data from the long-term grassland afforestation site at Glendhu confirmed that enhanced mineralisation of soil organic P occurred during the early stages of forest development (< 15 years after tree planting).

However, between 15 and 28 years after establishment soil organic P did not change while inorganic P decreased significantly. This suggested that trees were becoming increasingly reliant on accessing inorganic forms of P to sustain continued growth, which in turn may be linked to changes in soil organic P towards more recalcitrant forms with time under first rotation plantation forest. The latter is consistent with findings from the Orton Bradley Park temporal study.

In the long-term silvopastoral field trial at Lincoln widely-spaced *P. radiata* and grazed pasture were established simultaneously on high fertility/low organic matter soil previously managed under intensive mixed cropping. Changes in soil P observed during the first 10 years of the trial revealed significant increases in organic P, which was contrary to enhanced mineralisation of organic P observed when trees were planted in low fertility/high organic matter soils (at Orton Bradley Park and Glendhu). This suggests that pasture development and grazing had greater impacts on soil P dynamics than tree development leading to net P immobilisation. Changes in the management of the trial after 2000 meant that over the following 10 years tree growth gradually dominated over pasture which resulted in the decline and eventual elimination of the pasture understory. However, no net changes in soil P were observed over this period, although it is expected that significant changes in soil P may occur as the forest reaches maturity, over the 10 to 15 years following sampling in 2010.

Data from soils collected from the Haast chronosequence showed that rapid dissolution of primary mineral inorganic P occurred during the early stages of forest development with concomitant increases in soil organic P (i.e. P immobilisation). Accordingly, organic forms of P were predominant across the chronosequence and decreased with time due mainly to continued depletion of P by leaching. Results from the glasshouse experiment showed that the short-term bioavailability of soil organic P was similar for *P. radiata* compared with *C. macrocarpa*, which was consistent with results obtained from the Orton Bradley Park studies.

8.2 General Conclusions

The findings of this research project revealed that dramatic changes in the dynamics and bioavailability of soil P occurred in the first 5 years following the planting of trees in hill country land developed under improved grazed pasture. These changes showed that the cessation of grazing together with P acquisition and uptake by emerging tress caused substantial and significant depletion of soil organic P resources by enhanced mineralisation. It is likely that a significant proportion of the organic P in the pasture soils accumulated as a direct results of increased pasture productivity associated with inputs of P fertiliser over many years, and so the mineralisation of soil organic P by newly established trees effectively represents enhanced utilisation of accumulated nutrient reserves. This in turn represents enhanced nutrient efficiency and sustainability of marginal land-use in hill and high country areas of New Zealand. The impact of trees on organic P was also shown to be influenced by a combination of land-use history, soil fertility and plantation forest type, as shown by the significant increase in soil organic P that occurred following simultaneous establishment of trees and grazed pasture on land developed under high input mixed cropping. Changes in the dynamics and bioavailability of soil P following afforestation and between seasons were similar under contrasting forest tree species was a surprising finding to emerge from the Orton Bradley Park field trial studies. This indicated that factors other than differences in plant P uptake, litter return and mycorrhizal association did not have a major impact on P cycling during the initial stages of plantation forest development. However, a shift toward more recalcitrant forms of soil P with time under forest was attributed to the increasing influence of organic matter and nutrient inputs and turnover associated with continued tree growth and forest development. It is therefore possible that differences in the dynamics and bioavailability of soil P may emerge with time between the different tree species included in the Orton Bradley Park field trial.

8.3 Future Research

Herewith a list of potential topics for further research based on the findings of the current study:

1. It is important to continue detailed studies of the dynamics and bioavailability of soil P in monitored field plots and long-term trials in order to advance understanding of changes with time in different forest environments.

2. More investigation on the accumulation, nature and dynamics of soil organic matter in developing forest environments how this affects the cycling and bioavailability of P and other nutrients.

3. It is important to investigate the impacts of harvesting and replanting of short rotation plantation forests on soil organic matter and nutrient dynamics.

4. More studies are required to investigate the role and function of soil microbial communities, mycorrhizal fungi and root exudates on organic matter and nutrient cycling in plantation forests under different species.

5. Further development and application of molecular biology methods are required to explain links between microbes and function in the soil-plant environment.

Finally, it is important to suggest that future research focused on the temporal changes associated with afforestation should take into account other factors that could affect soil P dynamics and bioavailability with time, particularly changes in bulk density and spatial variability.

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